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Comptes rendus du vingtième colloque annuel de toxicologie aquatique: 17-21 octobre 1993, Québec, Québec

Proceedings of the Twentieth Annual Aquatic Toxicity Workshop, October 17-21, 1993, Quebec City, Quebec

ÉDITEURS/EDITORS

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Rapport technique canadien des sciences halieutiques et aquatiques 1989

Canadian Technical Report of Fisheries and Aquatic Sciences 1989

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sciences halieutiques et aquatiques 1989

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Cat. No. Fs 97-6/1989
ISSN 0706-6457

On devra citer la publication comme suit:

van Coillie, R., Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel et/and C. Thellen (eds.). 1994. Comptes rendus du vingtième colloque annuel de la toxicologie aquatique, 17-21 octobre 1993, Québec, Québec. Rap. Tech. can sci. halieut. aquat. 1989: 331 p.

©Minister of Supply and Services Canada 1994

Cat. No. Fs 97-6/1989
ISSN 0706-6457

Correct citation for this publication:

van Coillie, R., Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and/et C. Thellen (eds.). 1994. Proceedings of the Twentieth Annual Aquatic Toxicity Workshop, October 17-21, 1993, Quebec City, Quebec. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p.

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Ce volume renferme les textes et/ou les résumés brefs ou élargis de toutes les présentations à l'Atelier. Une table des matières et une liste des participants sont aussi incluses. Les textes et les résumés ont été redactylographiés et ensuite revus sommairement par les éditeurs. Toute remarque concernant les textes et/ou résumés devrait être adressée aux auteurs. Toutes les déclarations et les opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

This volume contains papers and/or abstracts or extended abstracts of all presentations at the Workshop. A table of contents and a list of participants are also included. The papers and abstracts were retyped and submitted to limited review by the editors. Comments on any aspects of individual contributions should be directed to the authors. Statements of view presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

PRÉFACE/ PREFACE

Le 20e colloque annuel de toxicologie aquatique a eu lieu au Château Frontenac à Québec du 17 au 21 octobre 1993. L'atelier a regroupé 13 séances plénières, 88 exposés, 56 affiches et 7 activités parallèles. Il y eut une participation de 367 personnes dont plusieurs conférenciers invités internationaux et nationaux.

Le 20e colloque annuel de toxicologie aquatique continue une série de discussions tenues annuellement au Canada sur les méthodes de mesures de toxicité et les parcours des contaminants dans l'environnement aquatique.

En 1973, les participants au 1er colloque se limitaient à quelques douzaines de fonctionnaires fédéraux soucieux de comparer des méthodes de bioessais. En 1993, la participation au colloque s'est élargie et a atteint quelques centaines de personnes des milieux gouvernementaux, industriels, universitaires et de la consultation, issus de divers pays. Les sujets traités se sont également diversifiés au cours de ces années. Les biotests continuent à susciter un intérêt évident mais des perspectives plus larges s'y ajoutent comme par exemple les biomarqueurs, l'évaluation de risque et le devenir des substances toxiques.

En cette période difficile, l'organisation d'un tel évènement exige la participation financière de plusieurs organisations. Nous remercions vivement les différents parrains qui nous ont aidés à surmonter ce défi et permis d'apporter plusieurs innovations.

Ces colloques sont planifiés par un comité international de direction. Leurs comptes rendus sont publiés avec le support du ministère des Pêches et Océans du Canada.

The 20th Annual Aquatic Toxicity Workshop was held at the Château Frontenac in Quebec city on October 17-21, 1993. The Workshop included 13 plenary presentations, 88 platform papers, 56 posters and 7 parallel activities. Total attendance was 367 and includes several international and national invited speakers.

The 20th Annual Aquatic Toxicity Workshop was on of a continuing series of annual workshops in Canada on toxicity testing methods, and pathways of contaminants in the aquatic environment.

In 1973, participation in the 1st workshop was limited to a few dozen public servants wishing to compare methods used for bioassays. In 1993, participation has expanded to include hundreds of scientists from governments, industries, universities and private consulting companies, coming from different countries. Topics have also evolved over the intervening years. Toxicity testing is still of great interest, but broader perspectives such as biomarkers, risk assessment and the fate of toxic substances are now discussed.

In this period of financial restraint, the staging of this kind of event necessitates financial support from many organizations. The Committee is very grateful to the different sponsors who have contributed to make this event successful and innovative.

These workshops are run by an international steering committee. The proceedings are published with the department of Fisheries and Oceans Canada.

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Le Comité tient à rendre hommage à la participation appréciée du regretté SERGE FRIAR de l'Alcan, qui est subitement décédé au printemps 1993: nos pensées vont vers lui.

The Committee whishes to pay tribute to the significant contribution of SERGE FRIAR, of Alcan, who suddenly passed away last Spring. He remains in our memory.

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Dimension Environnement Ltée, Montréal
Domtar, Donnaconna
Domtar, Windsor
Groupe SM, Aménatech, Sherbrooke
Hydro-Québec, Vice-présidence communications et relations publiques, Montréal
Kronos Canada Inc., Varennes
Ministère de l'Environnement du Québec, Sous-ministériat au milieu industriel, Sainte-Foy
Ministère des Forêts du Québec, Québec
Produits Forestiers Daishowa Ltée, Québec
SNC-LAVALIN Environnement Inc., Montréal
Société d'ingénierie Cartier Ltée, Montréal
Université Concordia, Montréal
Université de Montréal, Groupe interuniversitaire en limnologie, Montréal
Université du Québec à Chicoutimi, Groupe de recherche en productivité aquatique, Chicoutimi
Université du Québec à Montréal, Laboratoire de toxicologie en environnement, Montréal
Université du Québec à Rimouski
Université du Québec à Trois-Rivières
Université du Québec, Siège social, Sainte-Foy
Université Laval, Groupe interuniversitaire de recherches en océanographie du Québec, Sainte-Foy

SUBVENTION/ GRANT

Ministère de l'Environnement et de la Faune du Québec, Sainte-Foy

CONFÉRENCIERS INVITÉS / INVITED SPEAKERS

Ambassade de France au Canada, Ottawa
Analex Inc., Laval
Groupe Environnement Shooner, Genivar, Loretteville
Hydro-Québec, Vice-présidence environnement, Montréal
Ministère de l'Environnement et de la Faune du Québec, Direction des laboratoires, Sainte-Foy
Université du Québec à Montréal,
chaire de l'environnement, Montréal

POCHETTES DU COLLOQUE / WORKSHOP ENVELOPES

Analex Inc., Laval
Communauté urbaine de Québec
Consultants Beak Ltée, Montréal
Desseau Environnement Ltée, Saint-Romuald
GDG Environnement et laboratoires, Trois-Rivières
Groupe Environnement Shooner, Genivar, Loretteville
Laboratoires Eco-CNFS Inc, Pointe-Claire
Roche Ltée, groupe conseil, Sainte-Foy

Université de Montréal, Faculté d'éducation permanente, Montréal

TRADUCTION / TRANSLATION

Alcan, Montréal
Environnement Canada, Direction de la protection de l'environnement, Montréal
Environnement Canada, Eco-Santé, Hull
Environnement Canada, Réseau canadien des centres de toxicologie, Hull
Environnement Canada, Équipe d'intervention Saint-Laurent, Montréal
Pêches et Océans Canada, Direction régionale du Québec, Québec
QIT Fer et Titane Inc., Sorel
Secrétariat d'État du Canada, Ottawa

LOGISTIQUE / LOGISTICS

Château Frontenac, Hôtel Canadien Pacifique, Québec

Croisières Navimex Inc., Québec

Environnement Canada, Centre Saint-Laurent, Montréal

Environnement Canada, Direction de la protection de l'environnement, Montréal

Hydro-Québec, Vice-présidence environnement, Montréal

Ministère de l'Environnement et de la Faune du Québec, Direction des laboratoires, Sainte-Foy

SNC - LAVALIN Environnement Inc., Montréal

Technologies Polycontrôles, Montréal

Transfert du 10e colloque de toxicologie aquatique, Edmonton 4 - 7 octobre 1992

Université du Québec à Trois-Rivières

Université du Québec, Institut national de la recherche scientifique (INRS-Eau), Sainte-Foy

Université du Québec, Siège social, Sainte-Foy

COMPTES RENDUS / PROCEEDINGS

Pêches et Océans Canada, Institut Bayfield, Burlington

PROGRAMME/PROGRAM

Lundi, 18 Oct. **AM/PM** **Monday, Oct. 18**

08h45 Introduction (R. VAN COILLIE)

09h00 **L'importance de la toxicologie aquatique pour la santé humaine**

Impact of aquatic toxicology on human health

A. NANTEL

Session	*Biodégradation des contaminants* Biodegradation of contaminants	*Evaluation écologique des substances désignées prioritaires par la LCPE* Ecological assessments of priority substances under CEPA	*Biomarqueurs en santé humaine et des écosystèmes* Biomarkers in Human & Ecosystem health
10h15	Introduction (R.Siron)	Introduction (R. Kent/ D.R.J. Moore)	Introduction (J.G. Bontoux)
10h20	» **Étude microbiologique de la dégradation de composés aromatiques polluants** BISAILLON J.G, R. BEAUDET & F. LÉPINE	» **The CEPA priority substances assessment program** McBAIN D.C, R. CHÉNIER, C. FORTIN & D.R.J. MOORE	Conférencier d'ouverture (30 min) Keynote speaker (30 min) » **Biomarkers in ecosystem health monitoring** METCALFE C.D
10h40	...suite / continued	» **Current framework used to conduct ecological assessments of priority substances under CEPA** MOORE D.R.J, R. CHÉNIER, C. FORTIN & D.C McBAIN	» Commentaires / Replies (30 min) M. LaFaurie P. Speer K.R. Munkittrick P. Vasseur P. Deschaux P.V. Hodson
11h00	» **Genetics and applications of the tod system** LAU P.C.K, Y. WANG, D. LABBÉ et al.	» **Predicting effects of chlorobenzenes on benthic biota: Constraints and approaches** ELLIOT B.	
11h20	» **Biodégradation des BPC par les bactéries périphytiques du fleuve St-Laurent** SYLVESTRE M, D. PLANAS & C. VANIER	» **Key issues in the ecological assessment of chloroethanes** JONES S.	
11h40	» **Biological transformation of sediment PCBs in the St.Lawrence near the Massena, NY, superfund site** SOKOL R.C, O.S. KWON, C.M. CHARLOTTE & G.Y. RHEE	» **Data gaps and issues associated with assessment of priority metals in Canada** PORTER E.L, R.A. KENT & M. LEWIS	» Discussion générale (40 min) Round table discussion (40 min)
Session	*Biodégradation des contaminants* Biodegradation of contaminants	*Évaluation écologique des substances désignées prioritaires par la LCPE* Ecological assessments of priority substances under CEPA	*Biomarqueurs en santé humaine et des écosystèmes* Biomarkers in Human & Ecosystem health
13h35	Introduction (R. Siron)	Introduction (R. Kent/ D.R.J. Moore)	Introduction (P.V. Hodson)
13h40	» **Biodégradation des hydrocarbures: Curiosité de laboratoire ou outil opérationnel?** B. TRAMIER	» **Challenges in the assessment of complex mixtures in the Canadian environment** CONSTABLE M	Conférence d'ouverture (30 min) Keynote speaker (30 min) » **Biomarqueurs pour l'évaluation de la santé humaine** HAGUENOER J.M
14h00	...suite / continued	» **Groundwater issues in the assessment of priority substances in Canada** SCHNEIDER U.A, R.A. KENT & E.L. PORTER	» Commentaires / Replies (25 min) J.G. Bontoux P. Ayotte A. Gilman M. Feeley S. England
14h20	» **Oil spill bioremediation studies in low-energy shoreline environments** LEE K. & G.H. TREMBLAY	» **Data quality issues for ecological assessments** BOERSMA D.	
14h40	» **Biodegradation of PCP and BTEX in a lab-scale fluidized bed bioreactor** LEI J. & J.L. SANSREGRET	» **Use of wildlife models in the assessment of priority substances in Canada** LLOYD K.M. & L. BROWNLEE	» Discussion générale (35 min) Round table discussion (35 min)

Mardi, 19 Oct. AM Tuesday, Oct. 19

Session	*Microbiotests* Micro-scale bioassays	*Mercure dans l'environnement aquatique nordique* Mercury in the northern aquatic environment	*Évaluation écotoxicologique de risque* Ecotoxicological Risk Assessment
08h30	Introduction (K. Lee)	Introduction (R. Schetagne)	Introduction (G.W. Suter)
08h40	» **The status and future of marine microscale toxicity tests** WELLS P.G.	» **Mercury in Swedish forest soils and waters – Assessment of critical load** JOHANSSON K.	» **Ecological risk assessment for the terrestrial environment** J.L. DURDA
09h00	» **Évaluation de la toxicité de composés pétroliers et d'agents de traitement avec le test sur P. phosphoreum** SIRON R. & É. PELLETIER	... suite / continued	... suite / continued
09h20	» **Le dépistage des substances génotoxiques dans les mélanges complexes à l'aide du SOS Chromotest** LEGAULT R.	» **Mercury methylation activity of sediments from Mississagi river reservoirs** RODGERS D.W.	» **A framework for the environmental risk assessment of contaminated sediments** T.M. DILLON
09h40	» **Living luminescent biosensors to monitor aquatic pollutants and determine their underlying mechanisms of toxicity** DUBOW M.S, A. GUZZO, S. BRISCOE et al.	» **Wetlands as important sources of methyl mercury to boreal forest ecosystems** ST-LOUIS V.L, J.W. RUDD, C.A.KELLY et al.	... suite / continued
10h20	» **Le test JAYLET (ou test micronoyau triton): un nouvel outil pour la détection in vivo de la génotoxicité des eaux douces** FERRIER V. L. GAUTHIER, C. ZOLL-MOREUX & J. L'HARIDON	» **Sources and fate of mercury in hydroelectric reservoirs of northern Québec** LUCOTTE M, C. HILLAIRE-MARCEL, A. MUCCI et al.	» **Concepts and methods for assessing ecological risks in a watershed context** S.M. BARTELL
10h40	» **Development of environmental impact assessment tests based on the exoenzyme activity of indigenous bacteria** LEE K. & R. LAROCQUE	» **Exposition au mercure de balbuzards élevés sur les territoires de la Baie James et de la Baie d'Hudson** DESGRANGES J.L, J. RODRIGUE, B. TARDIF et al.	... suite / continued
11h00	» **Algae as tools in aquatic toxicity tests** NALEWAJKO C.	» **Évaluation de l'exposition des pêcheurs sportifs québécois au méthyl mercure: Résultats préliminaires d'une étude pilote** GRENIER A.M, É. DEWAILLY & S. GINGRAS	» **ERA prospects for the end of the 1990's** G. W. SUTER
11h20	» **Applications pratiques avec microalgues pour l'évaluation des dangers de contaminants relargués en milieu aquatique** BLAISE C.	» **Indications of increased Hg inputs to northern Canadian lakes as a result of atmospheric fallout** LOCKHART W.L.	... suite / continued
11h40	» **Development and validation of a new, rapid and economical protozoan bioassay for pulp and paper effluents** GILRON G.L., D.H. LYNN & R.P. SCROGGINS	... suite / continued	» **Discussion**

Mardi, 19 Oct. PM Tuesday, Oct. 19

Session	*Microbiotests* Micro-scale bioassays	*Bioaccumulation des contaminants* Bioaccumulation of contaminants	*Évaluation des effets* Effects Assessment
13h35	Introduction (K. Lee)	Introduction (P. Couture)	Introduction (A. Mudroch)
13h40	» **Développements de méthodes pour les tests de toxicité sur les liquides et les sédiments en utilisant des invertébrés marins provenant des régions arctiques de l'Amérique du Nord** RIEBEL P.N. & R. GANDIA	» **Mercury bioaccumulation by fish and crabs in a Texas bay: a modeling approach** ENGEL D.W. & D.W. EVANS	» **A test of equipotency of internal burdens of nine narcotic chemicals using D. magna** PAWLISZ A. & R. PETERS
14h00	» **A Japanese medaka egg exposure protocol for assessing the presence of sediment-associated embryo toxicants** BALCH G.C., J.S. GOUDEY & C.D. METCALFE	» **Some aspects on the role of a marine microalga in the fate of tributyltin in marine waters** St-LOUIS R., É. PELLETIER, P. MARSOT & R. FOURNIER	» **Assessing the aquatic toxicity of mixtures: Can toxicant body residues advance the state of the art?** McCARTY L.S. & D. MACKAY
14h20	» **Selection of toxicity bioassays based upon organism sensitivity** GREENE J.C.	» **A laboratory and field-based determination of the elimination and uptake rate constants for PCBs in D. polymorpha** MORRISON H.L., R. LAZAR & G.D. HAFFNER	» **Modelling population exposure-response functions for use in environmental risk assessment** POWER M., D.G. DIXON & G. POWER
14h40	» **Application of sediment bioassays for the environmental assessment of ocean dump sites** TAY K.L., K.G. DOE, A.J. MacDONALD et al.	» **La macroautoradiographie: un outil pour les études de toxicologie environnementale** ROULEAU C., É. PELLETIER & H. TJÄLVE	» **A bioenergetic approach to chronic toxicity assessment** MONITA D.M.A., H.M. GRAY & R.W. DAVIES

Mercredi, 20 Oct. AM Wednesday, Oct. 20

Session	*Plan d'action Saint-Laurent* St.Lawrence River Action Plan	*Hydrocarbures aromatiques polycycliques* Polycyclic aromatic hydrocarbons	*Effluents industriels et gestion environnementale* Industrial effluents and environmental management
08h30	Introduction (R. DeLadurantaye)	Introduction (J. Gearing)	Introduction (R. Parker)
08h40	» **Indicateur CHIMIOTOX pour les effluents industriels** DUCHESNEAU G. & G. LEGAULT	• **PAHs in environmental samples from Kitimat Arm, B.C.** CRETNEY W.J. & D. GOYETTE	• **Qualité de l'eau brute pour l'alimentation en eau potable et perspectives d'avenir** THÉBERGE S, S. PRIMEAU, R. McCORMACK & Y. GRIMARD
09h00	» **Barème d'évaluation de l'écotoxicité potentielle des effluents industriels** BERMINGHAM N., C. BLAISE & D. BOUDREAULT	• **PAHs in sediments of isolated northern Canadian lakes** LOCKHART W.L, B.N. BILLECK, P. WILKINSON et al	• **Research requirements for the developement of Canadian water quality guidelines** BROWN D.J., R.A. KENT, P.Y. CAUX & S. WALKER
09h20	» **Métaux traces dans les sédiments du St-Laurent** LORRAIN S., R. CARIGNAN & K. LUM	• **PAHs in sediments of the St.Lawrence estuary** GEARING J.N, P.J. GEARING, M. NOEL & J.N. SMITH	• **Les objectifs environnementaux de rejet pour les sources ponctuelles et le cadre légal au Québec** SINOTTE M.
09h40	» **Sedimentary record, isotopic composition and burden of industrial lead in the Laurentian trough sediments** GOBEIL C.	• **13C/12C ratios of individual PAH from marine and estuarine sediments** O'MALLEY V.P, T.A. ABRAJANO, J.HELLOU et al	• **Review and summary on joint action toxicity to aquatic organisms** BUCHMAN M.F.
10h20	» **Distribution and relative toxicity of PCDDs, PCDFs and planar PCBs in the Laurentian trough sediments** LEBEUF M., C. GOBEIL, C. BROCHU & S. MOORE	• **In situ sediment bioremediation in Hamilton harbour and St.Marys river** MURPHY T, A. MOLLER, J. THACHUK et al.	• **Boiler blowdown toxicity reduction tests** RODGERS D.W.
10h40	» **Esquisse d'un réseau de surveillance écotoxicologique de la faune du St-Laurent** RODRIGUE J., J.L. DESGRANGES & L. CHAMPOUX	• **PAH analysis and ecotoxicological testing of Athabasca river water and sediment** BROWNLEE B.G, G.A. MacINNIS, B.J DUTKA et al	• **Acute toxicity of oil sands wastewater: a toxic balance** VERBEEK A.G., W.C. MacKAY & M. MacKINNON
11h00	» **Fish as indicators of heavy metal pollution in the St.Lawrence River** SLOTERDIJK H.H, J. BUREAU & A.M. PRUD'HOMME	• **Metabolism of PAHs by brown bullhead** SIKKA H.C, J. PANGREKAR, P. KOLE et al	• **Identification of the toxic constituents (TIE) in a BCTMP mill effluent** GOUDEY J.S., M. FRITH, J. HANSEN & M. BROWN
11h20	» **Relative MFO activities in caged white suckers exposed to diffuse contamination in the St.Lawrence river** BUREAU J., A.M. PRUD'HOMME & H.H. SLOTERDIJK	• **Aromatic DNA-adducts in beluga whales from McKenzie delta and gulf of St.Lawrence** RAY S.	• **Le suivi des écosystèmes aquatiques dans l'industrie papetière canadienne: Grandes lignes et orientations futures** RIEBEL P.N. & M. TYLER
11h40	» **Écologie des grands fleuves** ANGELIER É.	• **Use of the bacterial luminescence assay to delineate porewater contamination with PAH in an Alberta river** HAMILTON H.R, B.R. TAYLOR & J.S. GOUDEY	• **Environmental fate and effects of bleached pulp mill effluents: Follow-up studies on dioxin, EROD induction and the role of dietary pathways.** KLOEPPER-SAMS P., E. BENTON, L. STEEVES et al

Mercredi, 20 Oct. PM Wednesday, Oct. 20

	Microcosmes & Mésocosmes Microcosms & Mesocosms	*Biomarqueurs* Biomarkers	*Effluents industriels et gestion environnementale* Industrial effluents and environmental management
13h35	Introduction (K. Liber/ K.R. Solomon)	Introduction (P.G.C. Campbell)	Introduction (P. Hodson)
13h40	» **Design, development and construction of a field microcosm research facility** SOLOMON K.R., K.E. DAY & J. CAREY	» **MTs and trace metal partitioning in the Atlantic oyster C. virginica** ENGEL D.W.	» **Evaluation of the steroid biosynthetic capacity of ovarian follicles from white sucker exposed to bleached kraft mill effluent (BKME)** McMASTER M.E. G.J. VAN DER KRAAK & K.M. MUNKITTRICK
14h00	» **The IML benthocosm: a mesocosm facility for the study of the deep soft bottom benthic environments** SILVERBERG N. & J.M. GAGNON	» **Induction of a small metal-binding protein (MT) in response to cadmium exposure in Gammarus** BOWES M.J. S.S. TOBE & A.P. ZIMMERMAN	» **Behavioural responses of rainbow trout to industrial effluents** WESTLAKE G.F., L.J. NOVAK & K.M. MURPHY
14h20	» **Impact of hexazinone and metsulfuron methyl herbicides on plankton communities of a forest lake** THOMPSON D.G, S.B. HOLMES, D. THOMAS et al	» **Potencies of mixtures of PCDDs for inducing EROD activity in rainbow trout** PARROTT J.L. J.Y. WILSON & P.V. HODSON	» **Aquatic toxicity and environmental impact of chlorinated wastewater effluent discharges from four sewage treatment facilities in the Atlantic region** RUTHERFORD L.A, K.G. DOE, S.J. WADE & P.A. HENNIGAR
14h40	» **Effects of diflubenzuron on non-target invertebrates in littoral enclosures** LIBER K., S.L. O'HALLORAN, K.L. SCHMUDE & T.D. CORRY	» **L'intégrité biotique des écosystèmes fluviaux** RICHARD Y.	» **Sediment quality and related effects on benthic organisms near mine sites** PRAIRIE R.
15h15	**Remise des prix étudiants/ Student awards** B. PINEL-ALLOUL **Constitution pour les Colloques / Constitution for the Workshops** K.R. SOLOMON **Mot de clôture / Concluding remarks** R. van Coillie		

AFFICHES/ POSTERS

18 OCTOBRE - OCTOBER 18

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OIL BIODEGRADATION IN COLD SEAWATER -A MESOCOSM STUDY-

P.02 **PADROS J., SIRON R., PELLETIER E., DELILLE D.**
EFFETS D'UN NOUVEL AGENT DE TRAITEMENT SUR LA BIODÉGRADATION DU PÉTROLE EN MILIEU MARIN

P.03 **MERCIER A., PELLETIER E., HAMEL J.-F.**
METABOLISM AND SUBTLE TOXIC EFFECTS OF BUTYLTIN COMPOUNDS IN STARFISH

P.04 **VANIER C., PLANAS D., SYLVESTRE M.**
DEVENIR DES BIPHÉNYLS POLYCHLORÉS DANS LES SÉDIMENTS

BIOACCUMULATION/BIOACCUMULATION

P.05 **GAGNON C., VAILLANCOURT G.**
ÉTUDE DE LA CINETIQUE D'ACCUMULATION DU CADMIUM PAR UNE MOUSSE AQUATIQUE, *FONTINALIS DALECARLICA* SCHIMP. EX. B.S.G., EN LABORATOIRE

P.06 **GÉLINAS S.,TESSIER L., PARIS S.,PAZDERNIK L.**
ÉTUDE DE L'ACCUMULATION DE MÉTAUX ESSENTIELS (Ca, Cu, Fe, Mg, Mn, Zn) CHEZ LE GASTÉROPODE *VIVIPARUS GEORGIANUS* (LEA)

P.07 **MUNGER C., HARE L.**
RELATIVE IMPORTANCE OF WATER AND FOOD AS TRACE METAL SOURCES TO LARVAE OF THE AQUATIC INSECT CHAOBORUS (DIPTERA)

P.08 **TESSIER L., VAILLANCOURT G., PAZDERNIK L.**
ÉVALUATION *IN VITRO* DE LA CINÉTIQUE DU CADMIUM ET DU MERCURE CHEZ DES MOLLUSQUES D'EAU DOUCE

P.09 **TESSIER L., VAILLANCOURT G., PAZDERNIK L.**
L'ABSORPTION DES MÉTAUX LOURDS CHEZ DES MOLLUSQUES SELON L'ÉQUATION D'ARRHÉNIUS

P.10 **TESSIER L., VAILLANCOURT G., PAZDERNIK L.**
MODELES DE PRÉDICTION DES TENEURS EN CADMIUM ET MERCURE CHEZ DES MOLLUSQUES D'EAU DOUCE

P.11 **ARSENAULT J.T., FAIRCHILD W.L.**
COMPARISON OF HEAVY METAL CONTENTS BETWEEN 1989-1991 AND 1993 IN SMELT AND IN SELECTED BY-CATCH SPECIES COLLECTED FROM THE MIRAMICHI ESTUARY

P.12 **GRANIER L.,TWISS M.R.,CAMPBELL P.G.C.,LAFRANCE P.**
ACCUMULATION DE DEUX MICROPOLLUANTS ORGANIQUES PAR L'ALGUE PICOPLANCTONIQUE SYNECHOCOCCUS : EFFETS DE LA MATIERE ORGANIQUE DISSOUTE ET DU pH

BIOGÉOCHIMIE/BIOGEOCHEMISTRY

P.13 **ROY R., CAMPBELL P.G.C.**
ALUMINUM COMPLEXATION BY A NATURALLY OCCURRING FULVIC ACID : DETERMINATION OF CONDITIONAL STABILITY CONSTANTS AND PREDICTION OF INORGANIC Al LEVELS IN SYNTHETIC ACIDIC SOFT WATERS

P.14 **WELSH P.G., DIXON D.G., RALPH K.M., MIERLE G.M.** CHRONIC TOXICITY OF COPPER TO LARVAL FATHEAD MINNOWS IN SOFT WATER

P.15 **YANKOVICH T.L., EVANS R.D.**
PREDICTIVE QUANTIFICATION OF TRACE METAL DYNAMICS AND PARTITIONING IN PARTICULATE MATTER

P.16 **WARREN L., OUTRIDGE P., ZIMMERMAN A.**
GEOCHEMICAL PARTITIONING OF COPPER IN AN ARTIFICIAL OXIDE-ORGANIC SEDIMENT

P.17 **SHTAMM E.V., SKURLATOV YU.I.**
TOXICITY CAUSED BY DISBALANCE OF REDOX PROCESSES IN NATURAL WATERS

P.18 **WAITE D.T., BANNER J., GURPRASAD N., CESSNA A.**
A NEW SAMPLER FOR COLLECTING DRY ATMOSPHERIC DEPOSITIONS OF ORGANIC CHEMICALS

P.19 **ROD'KO I.YA., SHTAMM E.V., SKURLATOV YU.I.**
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P.20 **HOUNTIN J., COUILLARD D., SIMARD R.R., GANGBAZO G., COTÉ D., LAFRANCE P.**
APPORTS RÉPÉTÉS DE LISIERS ET PROPRIÉTÉS CHIMIQUES D'UN SOL LOAM ARGILEUX NEUBOIS

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P.22 **KADLEC M.J., BUSH B.**
BIOCONCENTRATION FROM WATER AND BIOACCUMULATION FROM FOOD OF PCB CONGENERS IN RAINBOW TROUT (*ONCORYNCHUS MYKISS*) SUSPENDED IN CAGES IN THE ST. LAWRENCE RIVER FOR 30 DAYS

P.23 **KINNEY E., BUSH B.**
USE OF TRITURATE FROM MACROPHYTE SETTLED PARTICULATES FOR CONGENER SPECIFIC BIOMONITORING OF POLYCHLORINATED BIPHENYL (PCB) LEACHATE

P.24 **PHAM T., LUM K., LEMIEUX C.**
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P.27 **RODRIGUE J., DesGRANGES J.L., TITMAN R.**
USE OF PEKING DUCKS (*ANAS PLATYRHYNCHOS*) AS A BIOINDICATOR OF CONTAMINATION BY BIOACCUMULABLE SUBSTANCES IN THE NATURAL ENVIRONMENT (ST. LAWRENCE RIVER, CANADA)

P.28 **PINEL-ALLOUL B., MÉTHOT G., LAPIERRE L., WILLSIE A.**
COMPOSANTES ÉCOLOGIQUES ET TOXICOLOGIQUES DE LA STRUCTURE DU MACROBENTHOS : ÉTUDE DE CAS AU LAC SAINT-FRANÇOIS (QUÉBEC)

P.29 **FLESSAS C., PINEL-ALLOUL B., CAMPBELL P.G.C.**
ANALYSE DES FACTEURS BIOTIQUES ET ABIOTIQUES INFLUENÇANT LA BIOACCUMULATION DES MÉTAUX LOURDS CHEZ *BITHYNIA TENTACULATA* (MOLLUSCA : GASTROPODA) DU LAC SAINT-LOUIS

19 OCTOBRE - OCTOBER 19

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ALTÉRATIONS DES NIVEAUX DE CALCIUM CYTOSOLIQUE LIBRE ET DU pH INTRACELLULAIRE CHEZ LES HÉPATOCYTES DE TRUITE PAR LE TRI-n-BUTYLÉTAIN (TBT): ANALYSE PAR CYTOMÉTRIE DE FLUX

P.31 **MORIN P.E., AVERILL D., DENIZEAU F.**
EFFET D'UN STRESS THERMIQUE SUR LA VIABILITÉ ET LE Ca^{+} LIBRE INTRACELLULAIRE CHEZ DES HÉPATOCYTES DE TRUITE ARC-EN-CIEL

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Responsable/Convenor: Landis Hare, Université du Québec, Institut national de la recherche scientifique, INRS-Eau, Sainte-Foy.

RÉSUMÉS DES SESSIONS/ SESSION SUMMARY

BIODÉGRADATION DES CONTAMINANTS

Lundi, le 18 octobre 1993, A.M. et P.M.

Quels que soient les systèmes aquatiques (eaux souterraines, eaux de surface, milieux estuarien/marin, effluents), les processus biotiques jouent un rôle important dans la dégradation des contaminants organiques et par conséquent dans la réduction de la toxicité des milieux pollués. De la minéralisation aérobie des hydrocarbures monoaromatiques et des HAP à la fermentation des composés phénoliques ou à la déshalogénation des BPC dans les sédiments, la dégradation microbienne utilise de multiples voies métaboliques selon les conditions ambiantes et les polluants. La sélection de souches bactériennes spécifiques, les améliorations génétiques ou le développement de bioréacteurs permettent maintenant d'accroître l'efficacité des biotraitements d'effluents industriels. D'autre part, une meilleure connaissance de la biodégradation naturelle a conduit au développement et au succès des programmes de biorestauration de sites contaminés, en particulier à la suite de déversements pétroliers. Enfin, un processus moins connu mais tout aussi passionnant est la dégradation de certains contaminants organo-métalliques résultant du métabolisme de macro-organismes. A travers ces divers aspects de la biodégradation de composés xénobiotiques, cette session donnera l'occasion aux chercheurs concernés de discuter et de faire le point des recherches actuelles sur le sujet.

BIODEGRADATION OF CONTAMINANTS

Monday A.M. and P.M. October 18 1993

Whatever the aquatic system (groundwater, surface water, estuarine/marine waters, wastewater), biotic processes play an important role in the degradation of organic contaminants and further in the toxicity reduction of polluted environments. For example, from the aerobic mineralization of monoaromatic hydrocarbons and PAH's to the fermentation of phenolic compounds or the dechlorination of PCB's in sediments, microbial degradation involves numerous metabolic pathways according to the ambient conditions and the pollutants. The selection of specific bacteria, genetic improvements or the development of bioreactors now allow increased effectiveness in the biotreatment of industrial wastewaters. On the other hand, a better knowledge of natural biodegradation processes has led to the successful development of bioremediation programs for contaminated sites, particularly after oil spills. Finally, a less known but exciting process is the degradation of some organometallic contaminants resulting from macroorganism metabolism. Through these various aspects of the biodegradation of xenobiotics, this session will give the opportunity to scientists concerned with the topic to discuss and evaluate current research.

Responsable *Robert Siron*
Convenor *Université du Québec, Institut national de la recherche scientifique,*
INRS-Océanologie, Rimouski

ÉVALUATION ÉCOLOGIQUE DES SUBSTANCES DÉSIGNÉES PRIORITAIRES PAR LA LCPE

Lundi, le 18 octobre 1993, A.M. et P.M.

La Loi Canadienne de Protection de l'Environnement (LCPE) prévoit que le gouvernement fédéral, d'une part, réalise des évaluations écologiques sur les substances désignées prioritaires en vue de déterminer si celles-ci causent ou peuvent causer des effets néfastes à l'environnement. Des évaluations écologiques ont été complétées pour 44 substances dont des composés organiques volatiles, des organochlorés, des métaux, des mélanges et des effluents. Cette session se penchera sur le Programme d'évaluation des substances désignées prioritaires, sur le cadre d'évaluation, ainsi que sur les questions scientifiques que le programme a dû surmonter à ce jour. Les différentes collaborations avec la communauté scientifique seront décrites, et les avenues possibles pour améliorer ces liens seront examinées au cours d'une discussion libre à la fin de la session.

ECOLOGICAL ASSESSMENTS OF PRIORITY SUBSTANCES UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA)

Monday A.M. and P.M. October 18 1993

The Canadian Environmental Protection Act (CEPA) requires the federal government, in part, to conduct ecological assessments on priority substances to determine whether they cause or may cause harmful effects to the environment. Ecological assessments have been completed for 44 substances that includes volatile organic compounds, organochlorines, metals, mixtures and effluents. This session will describe the Priority Substances Assessment Program, the assessment framework, and the many scientific issues that have confronted the program to date. Current linkages with the scientific community will be described, and possible ways to enhance these linkages will be examined during an open panel discussion following the session.

Responsables *R.A. Kent*
Convenors *Eco-Health Branch, Environment Canada, Hull*
D.R.J. Moore
Commercial Chemicals Branch, Environment Canada, Hull

BIOMARQUEURS EN SANTÉ HUMAINE ET DES ÉCOSYSTEMES

Lundi, le 18 octobre 1993, A.M. et P.M.

Cette «Table-Ronde» vise à faciliter les échanges d'idées entre des toxicologues du milieu de la santé humaine et de la santé des écosystèmes au sujet de l'exposition aux polluants. Les progrès récents dans le développement des biomarqueurs pour l'évaluation de la santé vont être présentés par deux conférenciers principaux. Chaque membre de la «Table Ronde» participera aux discussions, et l'auditoire pourra se joindre au débat en fin de journée.

BIOMARKERS IN HUMAN & ECOSYSTEM HEALTH

Monday A.M. and P.M. October 18 1993

This «Round-Table» will provide an opportunity for the exchange of ideas among toxicologists concerned with assessing the health of humans and ecosystems in response to the discharge of pollutants. Recent advances in the development and use of «biomarkers» in health assessments will be presented by two keynote speakers. The «Round-Table» participants will respond to these presentations, and the general audience will be invited to join the Discussion at the end of the day.

Responsables *Peter V. Hodson*
Convenors *Environment Canada, Burlington, Ontario*
Jean G. Bontoux
Université de Montpellier, Montpellier, FRANCE

2 conférences principales et «Table-Ronde» avec 11 participants
2 Keynote addresses and «Round-Table» with 11 participants

MICROBIOTESTS

Mardi, le 19 octobre 1993, A.M. et P.M.

L'intérêt pour le développement et l'application des microbiotests a augmenté considérablement dans le domaine de l'écotoxicologie aquatique au cours des 10 à 15 dernières années. Sachant que l'impact environnemental d'une substance toxique ne peut être prédit avec fiabilité à partir des tests utilisant des espèces indicatrices seulement, on reconnaît de plus en plus le besoin de développer des tests permettant d'évaluer l'impact à des niveaux multi-trophiques. Conduits avec des bactéries, des micro-algues, des protozoaires, de petits invertébrés et des cultures cellulaires eucaryotiques, les microbiotests, représentant un segment réaliste du biote, deviennent des outils essentiels dans les études écotoxicologiques. Simples et peu coûteux, les microbiotests sont utilisés régulièrement au laboratoire en vue de prioriser et de dépister des produits chimiques potentiellement toxiques. De plus, ces microbiotests s'avèrent prometteurs pour les évaluations d'impact environnemental, puisqu'ils peuvent détecter rapidement des polluants (organiques et inorganiques) dans des échantillons liquides/sédiments complexes . Le développement et l'écologie potentielle, les aspects génétiques, la biotechnologie et l'immunochimie de microbiotests seront présentés et discutés au cours de cette session.

MICRO-SCALE BIOASSAYS

Tuesday A.M. and P.M. October 19 1993

Interest in the development and application of micro-scale bioassays (microbiotests) has increased dramatically in the field of aquatic ecotoxicology over the past 10-15 years. With conclusive evidence supporting the premise that the environmental impact of a toxicant cannot be predicted accurately with tests using single indicator species, there is now a recognized need for the development of multi-trophic level impact assessment tests. Conducted with bacteria, micro-algae, protozoans, small invertebrates, and eucaryotic cell-cultures, micro-bioassay tests representing a realistic cross-section of the biota are becoming an essential part of ecotoxicological studies. Simple and cost-effective, micro-bioassay tests have been routinely employed in the laboratory to rank and screen potentially toxic chemicals. In addition, these tests hold great promise for environmental impact assessments, since they enable the rapid detection of toxic pollutants (both organic and inorganic) in complex liquid/sediment samples. The development and potential ecology, genetics, biotechnology, and immunochemistry are presented and discussed in this workshop session.

Responsable *Kenneth Lee*
Convenor *Institut Maurice-Lamontagne, Mont-Joli*
Pêches et Océans Canada

MERCURE DANS L'ENVIRONNEMENT AQUATIQUE NORDIQUE

Mardi, le 19 octobre 1993, A.M.

D'origine géologique, le mercure est naturellement présent dans l'air, l'eau, les sols, les animaux et les humains. Des études récentes ont démontré que les concentrations dans les milieux naturels ont augmenté de façon importante depuis l'avènement de l'ère industrielle, par le transport atmosphérique à longue distance. De plus, une relation de cause à effet a été établie entre la création de réservoirs et l'augmentation des teneurs en mercure dans les poissons. Une consommation importante de poissons à teneurs élevées en mercure peut être dangereuse pour les animaux et les humains. Cette session vise l'échange entre différents intervenants impliqués dans le dossier mercure sur des sujets aussi variés que le transport atmosphérique du mercure, son cheminement dans l'environnement, sa biogéochimie en réservoirs nordiques et son transfert aux consommateurs de poissons.

MERCURY IN THE NORTHERN AQUATIC ENVIRONMENT

Tuesday A.M. October 19 1993

Originally from geological sources, mercury is naturally present in air, soils, water, animals and Man. Recent studies have demonstrated that concentrations in natural environments have greatly increased since the industrial age as a result of atmospheric fallout. Moreover, a cause-and-effect relationship has been found between reservoir creation and increased mercury concentrations in fish. Regular consumption of fish with high mercury levels may be dangerous for animals and Man. This session will provide an opportunity to exchange views on a number of aspects of the mercury issue, such as atmospheric transport, fate and pathways in the environment, biogeochemistry in northern reservoirs and transfer to animals and Man.

Responsable *Roger Schetagne*
Convenor *Hydro-Québec, Vice-président environnement, Montréal*

ÉVALUATION ÉCOTOXICOLOGIQUE DE RISQUE

Mardi, le 19 octobre 1993, A.M.

On a assisté au cours des dernières années à une évolution fulgurante du développement et de l'application de l'évaluation écotoxicologique de risque (EER). Cette session vise justement à faire le point sur les concepts et les approches les plus récents de cette discipline. Elle s'adresse aux praticiens de l'EER voulant parfaire leurs connaissances, aux scientifiques oeuvrant dans des domaines connexes et désirant contribuer à son développement et à son application, ainsi qu'aux gestionnaires devant intégrer l'EER à leur processus décisionnel. Les conférenciers aborderont le développement et l'application de ces méthodes d'évaluation pour des problématiques reliées aux milieux terrestre, aquatique et sédimentaire, individuellement ou en combinaison. Une attention particulière sera accordée aux perspectives de développement de cette discipline.

ECOTOXICOLOGICAL RISK ASSESSMENT

Tuesday A.M. October 19 1993

Ecotoxicological risk assessment (ERA) has evolved rapidly in recent years, both at the conceptual level and in the applications area. The aim of this session is to review the most recent concepts and approaches in this field - it has been designed to appeal to ERA practitioners interested in learning of the current state-of-the-art, to scientists working in related areas who may wish to contribute to the development and application of the ERA approach, and to environmental managers who need to incorporate ERA into their decision making process. The speakers will address the development and application of these methods to problems in the terrestrial, aquatic and sedimentary environments, considered separately or jointly. Particular emphasis will be accorded to the perspectives for future developments in this field.

Responsable *Louis Martel*
Convenor *Ministère de l'Environnement et de la Faune du Québec,*
Direction des laboratoires, Sainte-Foy

PLAN D'ACTION SAINT-LAURENT

Mercredi, le 20 octobre 1993, A.M.

Durant 5 ans, 1988 -1993, le Plan d'action Saint-Laurent I a initié plusieurs développements en écotoxicologie pour le suivi des contaminants dans le milieu aquatique. Entre autres, des outils ont été mis au point pour une évaluation «multimédia» de l'écotoxicité des mélanges de contaminants déchargés par les effluents industriels dans le fleuve, et pour un calcul de leur toxicité potentielle. Le devenir des contaminants est également examiné, notamment leur dépôt sédimentaire et leur bioaccumulation. Leurs effets biochimiques à court terme chez les poissons ainsi qu'un choix de bioindicateurs pour un réseau de surveillance écotoxicologique ont aussi été précisés.

ST. LAWRENCE ACTION PLAN

Wednesday A.M. October 20 1993

Over the past five years, 1988-1993, the St. Lawrence Action Plan (Phase I) has involved several ecotoxicological initiatives. Some of these initiatives, designed to monitor toxic contaminants in the St. Lawrence River system, will be discussed in this session. Tools have been developed for the «multimedia» evaluation of the ecotoxicity of contaminant mixtures discharged to the river in industrial effluents, and for the calculation of an index of their potential toxicity. The fate of contaminants has also been addressed, with particular emphasis on their sedimentation in the various fluvial lakes making up the St. Lawrence system and their bioaccumulation; the short-term effects of this bioaccumulation have also been studied, in fish. Finally, potential bioindicator species for an ecotoxicological monitoring network have been identified.

Responsable *Raymond Van Coillie*
Convenor *Environnement Canada, Direction de la protection, Montréal*

- effluents des colonnes XADpH7 et des colonnes XADpH2;
- extraits de filtration aux concentrations de 32 et 100 ml équivalents/litre;
- extraits de colonnes XADpH7 et XADpH2 aux concentrations de 32 et 100 ml équivalents/litre.

La durée du traitement a été de 12 jours dans tous les cas.

d - Effluents d'une unité de production de pâte à papier

Les eaux usées de ce site ont été testées à deux périodes différentes (mars 1991 et octobre 1991). Entre ces deux campagnes de prélèvement est intervenue une modification du procès de blanchiment de la pâte à papier (passage du blanchiment au chlore au blanchissement à l'oxygène).

Quatre types d'échantillons ont été testés:

- l'effluent brut en sortie d'usine (après lagunage);
- le panache de l'effluent dans la rivière voisine 50 m environ en aval du point de rejet;
- l'échantillon rivière aval 2000 m après l'usine.

À l'exception de l'effluent qui a dû être dilué de moitié, les autres échantillons ont pu être testés à l'état brut. La durée d'exposition a été de 12 jours dans tous les cas.

e- Eaux de la rivière Saudrune

La Saudrune est un petit effluent de la Garonne, dont le confluent se situe à l'entrée de la ville de Toulouse. Le cours inférieur de la rivière collecte, outre les eaux issues d'une des stations de traitement des eaux usées de Toulouse, celles d'un collecteur d'eaux pluviales desservant la zone sud de la ville et diverses zones industrielles (ateliers de traitement de surfaces et de textiles, industries chimiques). Les eaux de la Saudrune sont, à ce niveau, considérées comme de mauvaise sinon très mauvaise qualité pour divers paramètres (notamment azote ammoniacal, phosphates, nitrates, cadmium, détergents anioniques). Elles ont pu toutefois être testées à l'état brut (novembre 1991). La durée d'exposition a été de 12 jours.

RÉSULTATS

1. Tanneries

Le test réalisé en octobre 1985 montre une augmentation significative du taux d'érythrocytes micronuclées chez les larves exposées aux eaux du Dadou contaminées par les eaux usées des tanneries. Cette augmentation est proportionnelle à la concentration de l'effluent dans le milieu à l'essai. Quatre ans plus tard, l'effet génotoxique n'est plus détectable (fig. 1).

2. Raffinerie

Une induction de micronoyaux a été observée chez les larves soumises à l'effluent dilué (250 ml/litre). Les échantillons amont et aval, à la même concentration, n'ont pas présenté d'effet génotoxique. L'allongement de la durée d'exposition de 8 à 12 jours n'a pas modifié le résultat (fig. 2).

3. Industrie pétrochimique

a - Génotoxicité des eaux usées avant stérilisation

Les eaux usées du complexe pétrochimique ont entraîné une augmentation significative du taux d'erythrocytes micronucléées chez les larves de pleurodèle aux deux concentrations testées (32 et 100 ml/litre). Un effet dose net a été observé (fig. 3).

b - Génotoxicité des eaux usées après irradiation

La stérilisation des échantillons aux rayons γ ont entraîné une diminution de l'effet génotoxique, statistiquement significative toutefois uniquement à la concentration de 100 ml/litre (fig. 3).

c - Effets des procédés de concentration et d'extraction des composés organiques (filtration et adsorption sur résines XAD).

Les extraits de filtre ont entraîné un résultat positif aux concentrations de 32 et 100 ml équivalent/litre. Il en a été de même pour les extraits des colonnes excepté celui de la colonne XADpH2 à la concentration finale de 32 ml/litre (VAN der GAAG et *al.*, 1990). Un effet positif a été observé chez les larves exposées aux effluents des colonnes XADpH7 et XADpH2 (fig. 3). On peut en conclure que des substances génotoxiques subsistent dans l'eau après passage à travers les deux colonnes. Les analyses physico-chimiques effectuées par les auteurs de cette étude (VAN der GAAG *et al.*, 1990) ont d'ailleurs révélé qu'après les divers traitements, l'effluent contenait encore plus de 50% du carbone organique dissout et environ 30% de la fraction CaOX.

4. Papeterie

Aucun effet génotoxique n'a été observé chez les larves exposées aux divers échantillons testés, quels que soient les procès de blanchiment mis en oeuvre (fig. 4).

5. Rivière Saudrune

Les eaux de cette rivière se sont révélées légèrement génotoxiques à l'état brut (fig. 5).

DISCUSSION

Plusieurs travaux antérieurs avaient démontré que le test micronoyau triton est un modèle sensible et fiable pour la détection des substances génotoxiques présentes dans l'eau (SIBOULET *et al.*, 1984; GRINFELD *et al.*, 1986, JAYLET *et al.*, 1986a; JAYLET *et al.*, 1987; ZOLL *et al.*, 1988; FERNANDEZ *et al.*, 1989; GAUTHIER *et al.*, 1989; JAYLET *et al.*, 1990; GAUTHIER *et al.*, JAYLET et ZOLL, 1990; GAUTHIER et *al.*, 1990; L'HARIDON *et al.*, *1993;* FERNANDEZ et *al.*, 1993).

Les présents résultats confirment la capacité de ce test à détecter la présence de contaminants génotoxiques relargués dans l'environnement aquatique par divers sites industriels sous forme de mélanges complexes, même après dilution des échantillons testés. Des effets-doses peuvent être observés (tannerie, industries pétrochimiques).

Les premiers tests effectués en 1985 sur les eaux du Dadou polluées par le rejets de tanneries avaient été positifs. Le résultat négatif observé quatre ans plus tard peut être dû à plusieurs facteurs. D'une part, de gros efforts ont été entrepris entre temps en vue de réduire la pollution par une modernisation des procès de fabrication et le traitement des effluents avant leur rejet dans la rivière. Il en est résulté une baisse sensible du taux de certains micropolluants, notamment le chrome total. D'autre part, le prélèvement de 1989 a été réalisé après la connexion au collecteur des tanneries d'une fabrique de gélatine, entraînant une augmentation importante du débit de ce collecteur et par conséquent une dilution accrue des polluants génotoxiques en dessous du seuil de détection.

La même remarque peut être faite en ce qui concerne le résultat négatif obtenu avec l'effluent de la raffinerie de Martigues après sa dilution dans le canal latéral de l'étang de Berre 300 m en aval du point de rejet. Mais, on ne peut exclure la possibilité de réactions antagonistes entre certaines substances contenues dans l'effluent et des substances présentes dans le canal (par exemple liaison avec des substances chimiques ou adsorption sur des particules solides en suspension). La réponse positive obtenue avec l'effluent lui-même est probablement liée à la richesse en hydrocarbure lourds et aromatiques, détectés par analyse en spectrométrie de masse couplée à la chromatographie en phase gazeuse. Des expériences antérieures effectuées dans notre laboratoire ont établi la génotoxicité sur les larves de pleurodèle, d'hydrocarbures aromatiques polycycliques tels le benzo(a)pyrène, le pyrène, le benz(a)anthracène et le diméthylbena(a)anthracène à de faibles concentrations (respectivement 0.25, 0.175, 0.187 et 0.012 ppm) (JAYLET *et al.*, 1986a; FERNANDEZ et L'HARIDON, 1992).

Les résultats obtenus avec l'effluent pétrochimique néerlandais sont intéressants à plusieurs titres. Premièrement, ils démontrent l'aptitude du test JAYLET à détecter directement, sans traitement préalable, la génotoxicité d'un effluent pétrochimique, ce que n'ont pu faire, dans les conditions de l'étude (VAN der GAAG *et al.*, 1990) les tests bactériens mis en oeuvre (AMES et SOS Chromotest). Deuxièmement, tous les extraits testés (filtre, colonnes XADpH7 et pH2) se sont révélés génotoxiques *in vivo* chez les larves de pleurodèle au même titre qu'*in vitro* sur les tests bactériens (à l'exception de l'extrait de colonne XADpH2 à 32 ml/litre). Troisièmement, le test de JAYLET a permis de détecter directement une génotoxicité résiduelle dans l'effluent issu de la dernière colonne XADpH2, estimée par les auteurs de l'étude à 25% de la génotoxicité totale de l'effluent industriel brut.

On peut en conclure que, dans le cas de cette étude, le seul recours aux tests *in vitro* aurait conduit à une sous-estimation du potentiel génotoxique de l'effluent testé.

Aucune génotoxicité n'a été détectée avec le test JAYLET sur l'effluent de fabrique de pâte et papier. Cela confirme d'autres résultats récents, non encore publiés, obtenus dans notre laboratoire sur d'autres sites analogues français avec le même modèle biologique. Ils sont surprenants, surtout en ce qui concerne la première campagne de prélèvement (mars 1991) réalisée alors que le blanchiment de la pâte à papier au chlore et à l'hypochlorite de sodium était encore mise en oeuvre sur le site. Une analyse avait alors établi la forte teneur en chlore libre de l'effluent (200 ppm). Des résultats obtenus antérieurement dans notre laboratoire avaient démontré la clastogénicité du chlore et des composés chlorés sur les larves de pleurodèles (GAUTHIER, 1989: GAUTHIER *et al.*, 1989; GAUTHIER *et al.*, 1990). D'autre part, des études antérieures ont établi la génotoxicité de divers effluents de papeteries (MacGEORGE *et al.*, 1985; NESTMANN, 1985). Une étude réalisée au Centre Saint-Laurent de Montréal-Longueuil sur 15 effluents de ce type d'industrie a détecté la génotoxicité de 11 d'entre eux au moyen du SOS Chromotest appliqué à des échantillons bruts (COSTAN *et al.*, 1993). Il serait intéressant de conduire une étude comparative dans ce domaine, mettant en oeuvre divers tests de génotoxicité *in vitro* et le test JAYLET, du type de celle réalisée sur l'effluent pétrochimique néerlandais et discutée ci-dessus.

CONCLUSION

Les résultats présentés ici démontrent que les traitements appliqués aux mélanges complexes en vue de l'utilisation des tests *in vitro* (stérilisation, filtration, concentration et extraction) conduisent à la préparation d'extraits qui ne sont pas représentatifs de l'effluent brut initial. Le recours aux tests *in vivo* contournent cet inconvénient, qui pourra toutefois être levé par la mise au point de protocoles permettant l'application d'échantillons bruts ou dilués aux tests *in vitro* (Blaise, 1991). Cependant, même dans ce cas, les tests *in vivo* conserveront plusieurs avantages spécifiques. Premièrement, ils mettent en oeuvre des espèces aquatiques eucaryotes exposées directement aux effluents et phylogénétiquement plus proches de l'homme que les bactéries. Deuxièmement, la réaction de l'animal prend en compte les interactions qui se produisent entre divers micropolluants d'un mélange complet et leur biodisponibilité et elle résulte de son métabolisme général. En définitive, les réponses obtenues expriment non seulement les phénomènes complexes qui se déroulent dans l'organisme entier, mais aussi les interactions entre l'organisme et son environnement. Le test JAYLET, sensible et fiable, répond à ces critères. Il a toute sa place, aux côtés d'autres techniques *in vitro* et *in vivo*, dans la nécessaire batterie de tests à sélectionner en vue de l'évaluation la plus objective possible de l'impact génotoxique des pollutions antropogéniques sur les écosystèmes.

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LÉGENDES DES FIGURES

Histogrammes des médianes et de leurs intervalles de confiance au seuil de sécurité de 95%

Figure 1: Génotoxicité d'effluents de tanneries

L'effluent prélevé en 1985 induit chez le pleurodèle une augmentation du taux de micronoyaux avec effet-dose. L'effet négatif obtenu en 1990 est en relation avec l'amélioration des techniques de tannage et du traitement des eaux usées d'une part, à la dilution de l'effluent par celui d'une fabrique de gélatine d'autre part.

Figure 2: Génotoxicité d'effluents d'une raffinerie de pétrole

L'exposition pendant 8 jours de larves de pleurodèle aux effluents prélevés en mars (M) et novembre (N) 1990 provoque une augmentation significative du taux de micronoyaux. La prolongation du traitement jusqu'au 12ème jour (N-12) ne change pas le résultat. Après dilution dans le canal récepteur des eaux usées, l'effet génotoxique n'est plus détecté.

Figure 3: Génotoxicité d'un effluent d'usines pétrochimiques

Un effet positif accompagné d'un effet dose est observé aux deux dilutions testées. L'irradiation de l'échantillon diminue le potentiel génotoxique (à la plus forte concentration) sans la détruire totalement. Une génotoxicité résiduelle est encore détectée à la sortie des colonnes XADpH7 et XADpH2.

Figure 4: Cas des effluents d'une fabrique de pâte à papier

Lors des deux campagnes de prélèvement (91/3 et 91/10), aucun des échantillons testés (effluent, panache, aval) ne s'est révélé génotoxique sur les larves de Pleurodèle.

Figure 5: Génotoxicité de la rivière Saudrune

Les eaux brutes prélevées en novembre 1991 ont présenté à l'état brut une faible génotoxicité.

Figure 1

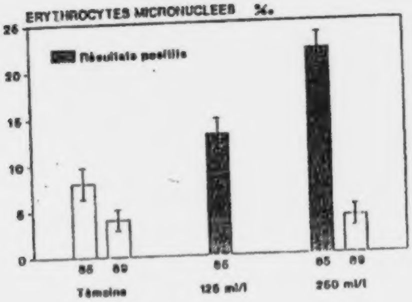


Figure 2

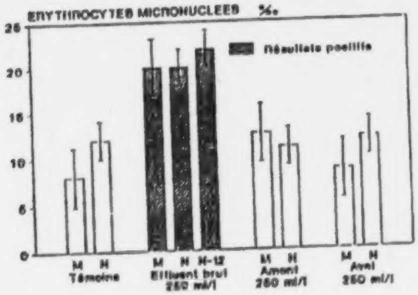


Figure 3

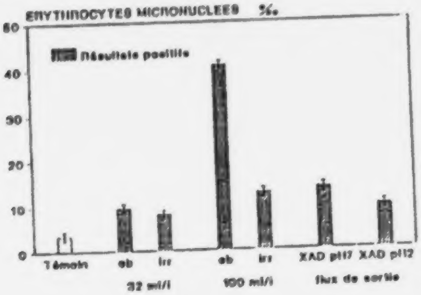


Figure 4

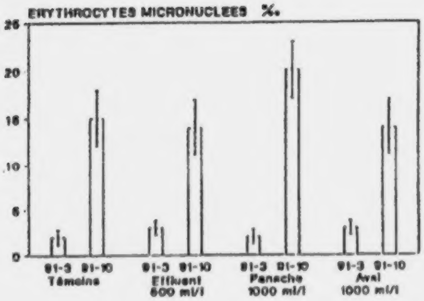
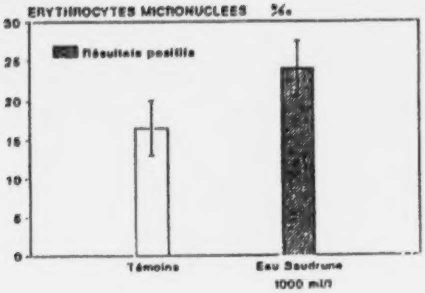


Figure 5



EFFETS D'UN NOUVEL AGENT DE TRAITEMENT SUR LA BIODÉGRADATION DU PÉTROLE EN MILIEU MARIN.

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RÉSUMÉ

Plusieurs régions de l'estuaire maritime du Saint-Laurent très riches d'un point de vue écologique sont considérés comme des zones "à haut risque" en cas de marée noire. Les méthodes traditionnelles de nettoyage et de revalorisation d'un milieu atteint par le pétrole sont non seulement coûteuses, mais aussi partiellement efficaces. Afin de remédier à ces problèmes, une famille de nouveaux produits anti-adhésifs a été développée au Centre Océanographique de Rimouski. Dans le cadre de l'évaluation écotoxicologique de ces nouveaux composés, l'objectif de cette étude expérimentale était d'évaluer l'action de la communauté bactérienne naturelle de l'Estuaire du Saint-Laurent sur le pétrole traité par le nouvel agent anti-adhésif et de comparer avec le pétrole non traité.

Un groupe de 4 mésocosmes de 3,5 m^3 chacun a été utilisé pour le suivi de la biodégradation des résidus pétroliers traités et non traités, ainsi que pour étudier l'évolution la flore bactérienne en période estivale pendant 62 jours. La biodégradation des résidus de surface a été suivie par chromatographie en phase gazeuse, à l'aide de plusieurs rapports d'hydrocarbures, dans les fractions aliphatiques et aromatiques. Dans chaque réservoir, les bactéries hétérotrophes viables (comptages sur plaques et par la technique de Most Probable Number), les bactéries spécifiques du pétrole (MPN) ainsi que les bactéries totales (comptages directs en épifluorescence) ont été régulièrement mesurées.

Dans les conditions simulées dans ce travail, le nouvel agent de traitement semble ne pas avoir d'effet sur le maillon bactérien. Le pétrole traité provoque une stimulation de l'activité hétérotrophe et des bactéries capables de métaboliser le pétrole, ainsi que de la biomasse bactérienne. Cette évolution de la flore bactérienne était tout à fait comparable à celle provoquée par le pétrole non traité. Le produit seul a également stimulé l'activité hétérotrophe. Ces résultats suggèrent que la réponse bactérienne à une contamination pétrolière n'a pas été affectée par le traitement. L'efficacité de ce nouveau produit à piéger le pétrole en surface a été reflétée par une forte réduction de l'évaporation des hydrocarbures (rapport C14/C28). D'autre part, le produit semble ne pas affecter la biodégradation (rapports n-alcanes/isoprénoides et pristane/phytane) ni l'altération (évaporation, photo-oxydation, activité microbienne de la fraction aromatique du pétrole.

Finalement, ces résultats préliminaires confirment que les mésocosmes sont d'excellents outils pour l'écotoxicologie expérimentale, permettant de mieux prévoir le devenir et les effets du pétrole traité par ces nouveaux agents chimiques suite à un accident pétrolier.

EXPOSITION AU MERCURE CHEZ DE JEUNES BALBUZARDS AU NID DANS LES TERRITOIRES DE LA BAIE-JAMES ET DE LA BAIE-D'HUDSON.

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RÉSUMÉ ALLONGÉ

Des données relatives à l'écologie et à l'exposition au mercure recueillies entre 1989 et 1991 sur 142 aiglons de balbuzard (*Pandion haliaetus*) appartenant à 82 nichées établies sur des lacs, des rivières et des réservoirs hydroélectriques nordiques, ont permis de mesurer l'importance et certaines des conséquences du transfert du mercure bioaccumulé par les poissons vers la chaîne alimentaire semi-aquatique représentée par cette espèce de rapace pêcheur.

Biodisponibilité accrue du mercure

Les teneurs en mercure que nous avons dosées dans les tissus des aiglons mettent en évidence la disponibilité accrue du mercure dans les jeunes réservoirs hydro-électriques (10 à 12 ans à LG2). En effet, les jeunes élevés en milieu naturel au Complexe La Grande ont en moyenne 0,3 mg/ kg de mercure dans le sang et 5,9 mg/kg dans leurs plumes (n = 29). Ces teneurs sont 6 fois supérieures chez les aiglons des milieux aménagés: 1,9 mg/kg dans le sang et 37,3 mg/kg dans les plumes (n = 79). On retrouve également des différences au moins aussi importantes entre les milieux, pour tous les autres tissus que nous avons analysés (foie, reins, muscles, cerveau). La quantité moyenne de mercure total dans un aiglon (poids moyen de 1,5 kg) élevé en milieu naturel est de 1,6 mg, comparativement à 10,5 mg pour les milieux aménagés. Des analyses de contenus stomacaux montrent que 90% de ce mercure est ingéré sous forme méthylique. Les concentrations sont très variables entre les stations des milieux aménagés. Ainsi, à LG4-Lanouette, les aiglons ne sont pas plus contaminés que ceux des milieux naturels: sang: 0,6 mg/kg; plumes: 10,8 mg/kg: n = 3. À l'opposé, ceux de LG2-Toto, affichent les niveaux de contamination les plus élevés: sang: 3,4 mg/kg; plumes: 71,1 mg/kg; n = 4.

Bioamplification du mercure dans le sang et les plumes

Nous avons modélisé le phénomène de bioamplification du mercure chez le jeune Balbuzard, en mettant en relation les concentrations dosées dans ses tissus, avec les quantités de mercure ingérées. Cette dernière variable a été calculée par l'intermédiaire d'un bilan des types et des tailles des proies consommées par le balbuzard à 21 stations (6 milieux naturels, 15 milieux aménagés), que nous avons mis en relation avec des données relatives à la contamination de la chair des poissons recueillis à ces mêmes stations dans le cadre du réseau de suivi environnemental du complexe La Grande.

La relation entre la teneur en mercure dans le sang des aiglons et la dose qui leur est servie par leurs parents est:

$$[Hg]\ \text{sang} = 1{,}09\ [Hg]\ \text{poissons} + 0{,}34$$

($R^2 = 0{,}68$; $p < 0{,}01$; $n = 21$). La pente de cette équation n'étant pas statistiquement différente de 1, la concentration du mercure dans le sang constitue donc une estimation valable de la concentration de mercure dans la nourriture ingérée par les aiglons. Compte tenu de ceci, nous posons que la relation:

$$[Hg]\ \text{plumes} = \Big(\big(78{,}09\ (2{,}21^{[Hg]\ \text{sang}})\big) \big/ \ (1{,}07^{\text{âge des aiglons}})\Big) - 1$$

($R_2 = 0,96$; $p < 0,01$; $n = 21$) est le reflet de celle unissant la concentration du mercure dans les plumes et celle dans les proies. Cette relation est plus complexe que la précédente puisque la concentration du mercure dans les plumes varie également en fonction de l'âge des aiglons. Contrairement à ce qui se passe dans le sang, cette relation met en évidence une bioamplification extrêmement importante depuis la dose ingérée vers les plumes. De plus, pour une dose de mercure donnée, l'intensité de cette biomagnification varie en fonction de l'âge des aiglons. La bioamplification est maximale au plus fort de la croissance des aiglons, soit au début de la formation des plumes de vol (20-25 jours) et diminue ensuite progressivement jusqu'à l'âge de 45 jours, lorsque la croissance des plumes de vol est complétée. Cette relation illustre clairement que la croissance des plumes protège l'aiglon puisqu'elles agissent comme un exutoire où est dirigé le mercure ingéré. Nous avons d'ailleurs calculé que, tant dans les milieux naturels qu'aménagés, environ 85% du mercure bioaccumulé se trouve dans le plumage. Une fois leur croissance complétée, les plumes ne sont plus irriguées par le sang de sorte que le mercure qu'elles contiennent ne peut plus être remis en circulation dans l'organisme.

Ces deux modèles de bioamplification, outre leur intérêt théorique, permettent de calculer avec beaucoup de justesse et économie de moyens, le niveau de contamination en mercure qu'on retrouve dans le milieu.

Conséquences de l'exposition au mercure chez le balbuzard

Bien que la quantité de mercure accumulée par les aiglons des réservoirs soit en moyenne beaucoup plus élevée que ce qu'on retrouve dans les milieux naturels, nous n'avons pas constaté de conséquence néfaste de cette contamination. Dans les 2 stations où l'exposition au mercure est la plus forte (LG2-Toto et LG3-Roy), on retrouve en moyenne 1,6 jeune par nichée à l'envol, ce qui est comparable à ce qu'on retrouve aux autres stations situées sur les réservoirs (1,8 jeune/nichée) ou en milieu naturel (2,0 jeunes/nichée). Considérant que la productivité minimale qu'une population de balbuzards doit maintenir afin de compenser la mortalité annuelle de ses adultes est de 0,8 à 1,2 jeune/nichée, les performances reproductrices que nous rapportons sont supérieures dans tous les cas. Incidemment, la densité des nids fréquentés est plus élevée en périphérie des réservoirs (2,1 nids/100 km de rivage) qu'en milieu naturel (1,3 nid/100 km).

Le stockage du méthylmercure dans les plumes en croissance fournit un excellent exutoire qui prévient l'accumulation du mercure dans les tissus internes. Des problèmes toxicologiques pourraient cependant exister après l'envol, une fois la croissance des plumes terminée. Selon la durée du séjour pré-migratoire des jeunes à proximité des sites de nidification, il pourrait y avoir une accumulation importante de mercure dans les organes vitaux. Ce phénomène devrait être examiné avant de conclure que l'exposition au mercure n'est pas suffisamment élevée chez les aiglons de réservoirs pour nuire à leur survie après l'envol.

Remerciements

Cette étude fut rendue possible grâce à l'appui financier de la vice-présidence Environnement d'Hydro-Québec.

ESQUISSE D'UN RÉSEAU DE SURVEILLANCE ÉCOTOXICOLOGIQUE DE LA FAUNE DU SAINT-LAURENT: LE RÔLE DU SERVICE CANADIEN DE LA FAUNE. Jean-Luc DesGranges, Jean Rodrigue et Louise Champoux. Environnement Canada, Service canadien de la faune, 1141, route de l'Église, Sainte-Foy, Québec, G1V 4H4, Canada

RÉSUMÉ ALLONGÉ

Un des principaux objectifs du Plan d'action Saint-Laurent étant la réduction de 90% de la charge polluante acheminée au fleuve par 50 usines prioritaires, il importe de développer un outil qui permette d'évaluer le succès des actions entreprises pour nettoyer le fleuve Saint-Laurent. Dans le cadre d'un comité d'harmonisation fédéral-provincial, des chercheurs de plusieurs agences gouvernementales (CSL, SCF, P&O, MLCP, MENVIQ) mettent actuellement au point un réseau de surveillance de la contamination des chaînes alimentaires du système du Saint-Laurent qui repose sur l'utilisation judicieuse de bioindicateurs. Ce réseau de bioindication doit permettre de suivre les variations spatio-temporelles de la contamination chez des espèces exposées différemment aux contaminants selon la niche et le niveau trophique qu'elles occupent. D'autre part, le réseau devra aussi permettre de suivre la récupération des diverses espèces actuellement affectées par la toxicité du milieu fluvial.

Le Service canadien de la faune a la responsabilité d'évaluer le potentiel de bioindication des amphibiens, des reptiles et des oiseaux qui habitent le Saint-Laurent (DesGranges et Thompson, 1990; DesGranges, 1992). Les espèces qui seront retenues doivent permettre de détecter des changements statistiquement significatifs tant au niveau des sources locales de contamination, qu'au niveau de la pollution diffuse dans le milieu récepteur.

Une première série de tests porte sur des espèces indicatrices d'exposition aux contaminants (métaux: Hg, Pb, Cd, As - composés organiques: pesticides organo-chlorés, BPC, PCDD, PCDF). Deux espèces invertivores qui vivent près des sources ponctuelles de contamination servent au suivi des contaminants dans le benthos [le Necture tacheté (*Necturus maculosus*), un amphibien] et les insectes émergents [l'Hirondelle bicolore (*Tachycineta bicolor*), un oiseau utilisant des nichoirs artificiels]. Plusieurs autres espèces sont évaluées quant à leur intérêt pour suivre la contamination régionale. L'Eider à duvet (*Somateria mollissima*, un molluscovore marin) renseigne indirectement sur la contamination de la matière en suspension tandis que plusieurs espèces invertivores et piscivores permettent de suivre les contaminants dans des organismes nectoniques d'âges, de tailles et de régimes alimentaires différentes. Ce sont: la Chélydre serpentine (*Chelydra serpentina*, une tortue), de même que la Guifette noire (*Chlidonias niger*), la Sterne pierregarin (*Sterna hirundo*), le Goéland argenté (*Larus argentatus*), le Cormoran à aigrettes (*Phalacrocorax auritus*), le Bihoreau à couronne noire (*Nycticorax nycticorax*) et le Grand Héron (*Ardea herodias*), des oiseaux coloniaux qui se nourrissent soit dans les milieux humides ou dans le fleuve lui-même.

Les espèces aquatiques (le Necture et la Chélydre) retenues résident en permanence dans le fleuve et se déplacent relativement peu au cours de leur existence. Dans le cas du Grand Héron, du Bihoreau à couronne noire, de la Sterne pierregarin et de l'Hirondelle bicolore, des oiseaux migrateurs semi-aquatiques, nous concentrons nos efforts à l'étude des jeunes davantage représentatifs de la contamination du fleuve.

Une deuxième série de tests porte sur des biomarqueurs physiologiques qui renseigneront sur la santé des organismes vivants dans le fleuve. Pour le moment, les études se limitent au Grand Héron et au Bihoreau à couronne noire. Certains des biomarqueurs étudiés indiquent une exposition à certains contaminants et avertissent de problèmes toxicologiques potentiels. Ce sont, entre autres: l'induction des MFO, les protéines fixatrices de métaux (MT), les porphyrines, la vitamine A et les hormones thyroïdiennes. D'autres biomarqueurs signalent des agressions toxiques plus importantes et susceptibles d'avoir des effets à plus long terme sur les organismes. Ce sont, entre autres: l'inhibition de l'ALA-D et les adduits d'ADN. D'autres biomarqueurs et d'autres espèces devront encore être évalués avant d'en arriver à une sélection définitive d'indicateurs de santé.

Par ailleurs, d'autres indices tels le profil métabolique du sang des jeunes, les performances reproductrices et le suivi des populations du Grand Héron donnent un aperçu de l'état général des individus et des populations de cette espèce sentinelle de la qualité des écosystèmes aquatiques du Saint-Laurent.

Bien que la sélection définitive des espèces bioindicatrices et des biomarqueurs physiologiques nécessite le sacrifice d'un certain nombre d'individus pour l'examen de leurs organes internes (foie, reins, cerveau, muscles), nous profitons de cette étude pour mettre au point des techniques de dépistage qui permettront de connaître la contamination et la condition de la faune à l'aide des plumes, du sang et d'un nombre minimal d'oeufs par couvée, des tissus qui n'exigent pas de tuer d'animaux.

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ÉVALUATION DU CANARD DE PÉKIN COMME BIOINDICATEUR DE LA CONTAMINATION EN MILIEU NATUREL. Jean Rodrigue[1], Jean-Luc DesGranges[1] et Rodger Titman[2] [1]Service canadien de la faune, C.P. 10100, Ste-Foy, Québec, Canada. G1V 4H5 [2] McGill University, 21111 Lakeshore, Sainte-Anne-de-Bellevue, Québec, Canada. H9X 1C0

RÉSUMÉ ALLONGÉ

Des canards de Pékin femelles (*Anas platyrhynchos*) adultes âgées de 18 mois ont été placées dans plusieurs milieux humides de la rivière des Outaouais (125 km de rivière) et de la portion d'eau douce du fleuve Saint-Laurent (375 km de fleuve), pour des périodes variant entre 14 et 73 jours durant les étés 1987, 1988 et 1989. Deux stations situées en milieu fluvio-lentique (lac Saint-François) qui présentaient des concentrations en BPC différentes dans les sédiments (Sloterdijk, 1985) ont été retenues pour développer une courbe d'accumulation temporelle des contaminants dans le foie. La récolte de quatre individus a été effectuée à intervalle régulier (15 jours en moyenne) à chacun de ces sites tout au long de l'étude.

Variation du poids corporel

Le poids des canards a rapidement diminué durant les vingt premiers jours passés en milieu naturel. Il s'est ensuite stabilisé après environ quarante jours, soit une vingtaine de jours après que les canards aient commencé à se nourrir des ressources alimentaires du milieu. Cette perte de poids est sans doute attribuable au stress causé par: le changement d'habitat (de la ferme d'élevage au milieu naturel), la dépense énergétique liée à la recherche de nourriture (ils étaient nourris *ad libidum* à la ferme d'élevage), celle entourant la mue [le coût direct du remplacement des plumes et le coût indirect de la thermorégulation (Farner et King, 1972; Norstrom et al., 1986)] et possiblement à l'ingestion de plombs de chasse (Sanderson et Bellrose, 1986).

Exposition aux contaminants

Une augmentation rapide du nombre de contaminants dans le foie a été observée chez les canards exposés. Le pourcentage de contaminants détectés augmente pour atteindre un maximum de 80% après un séjour d'environ 30 jours en milieu naturel. Un plateau se dessine par la suite entre 60% et 80%. Le nombre de contaminants détectés est fonction: du temps d'exposition des canards, de la contamination du site et de la capacité de métabolisation de ces composés par les canards.

Patron de bioaccumulation

Il n'y a pas de différence significative entre les courbes d'accumulation temporelle des contaminants dans le foie aux deux stations du lac Saint-François. L'accumulation durant les deux premières semaines de séjour en milieu naturel a été négligeable, les canards utilisant leur abondante réserve lipidique pour survivre. Une fois celle-ci fortement entamée, les canards recommencent à se nourrir d'aliments qu'ils trouvent dans le milieu naturel de sorte que les concentrations de contaminants commencent alors à augmenter dans le foie.

Même si les concentrations atteintes sont faibles (99.9% des 876 mesures d'OC-BPC sont inférieures à 1 mg/kg), l'accumulation des composés organochlorés et des BPC détectés a été très rapide. En effet, les individus séjournant en milieu naturel sont de 10 à 1000 fois plus contaminés que les témoins pour une période d'exposition de moins de 2 mois.

Potentiel de bioindication du Canard de Pékin

Le recours à des canards domestiques comme sondes bioanalytiques présente plusieurs avantages. On peut les obtenir facilement et à bon compte, tout en contrôlant le sexe et l'âge des individus. Étant peu mobiles et faciles à retrouver, ils nous apportent une information de qualité sur la contamination de sites particuliers. Il est entre autre possible de suivre la bioaccumulation de plusieurs xénobiotiques tels les métaux lourds et les composés organochlorés. L'utilisation de Canards de Pékin présente toutefois certaines limites et quelques désavantages comme la présence absolument nécessaire d'habitats adéquats permettant la survie des canards, de même que le problème du stress qu'entraîne le transfert de la ferme d'élevage au milieu naturel. Pour la première fois de leur vie, les canards doivent trouver eux-mêmes leur nourriture, ce qui les expose, pour la première fois aussi, à la prédation et au braconnage.

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ÉVALUATION DE LA QUALITÉ DES SÉDIMENTS PRÈS DE SITES MINIERS ET DES EFFETS SUR LES ORGANISMES BENTHIQUES.

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RÉSUMÉ ALLONGÉ

Les sédiments de milieux aquatiques situés à proximité de sites miniers peuvent montrer des signes d'enrichissement par les métaux lourds. Ces concentrations plus élevées sont reliées à la sédimentation de solides en suspension non captées par le système de traitement, ou à d'autres causes pouvant être reliées au drainage minier acide. Les effets de ce type de contamination sur la faune aquatique, particulièrement sur les organismes vivant dans ces sédiments (soit le benthos), peuvent être toutefois très variables.

Une revue de l'information disponible sur ce sujet pour plusieurs sites du groupe Noranda a été effectuée. Plus récemment, des études environnementales (effectuées dans le cadre de la préparation de plan de fermeture de mines pour les sites Mattabi Mines Ltd., et Minéraux Noranda Inc. Division Geco) ont permis d'examiner plus en profondeur différents aspects de la toxicité des métaux lourds dans les sédiments sur la faune benthique. Ainsi, les diverses concentrations de métaux dans les phases solide et liquide des sédiments ont été examinées en fonction des effets sur la structure de la communauté benthique ainsi que des effets toxiques sur des espèces benthiques spécifiques (Hyalella, Chironomus, Hexagenia).

Bien que les analyses chimiques effectuées ont inclus un nombre important d'éléments, le zinc a été utilisé comme principal traceur de la contamination des sédiments aux sites étudiés. Une forte corrélation (r^2>0.6) existe par ailleurs entre le zinc et les autres éléments principalement responsables de la contamination, comme le cuivre, le plomb et le cadmium. Afin de mettre en perspective le niveau de contamination par le zinc observés dans ces sédiments, il convient de décrire les critères de qualité des sédiments développés récemment par le Ministère de l'environnement de l'Ontario (MEO). Ainsi, les valeurs numériques pour le zinc prédisant des effets mineurs et des effets néfastes sur la faune benthique sont 120 µg/g et 820 µg/g respectivement.

L'éventail des concentrations de zinc observées dans les sédiments de surface de divers plans d'eau près de Mattabi échantillonnés en 1989 s'est avéré très large: de 33 à 43 800 µg/ g Zn (poids sec). Un nombre important de sites possédaient des concentrations supérieures au critères d'effets néfastes du MEO. Néanmoins, les effets sur la faune benthique évalués en fonction de ces concentrations étaient variables: par exemple, de 4 à 19 taxons benthiques pouvaient être rencontrés, et ce, à des concentrations supérieures au critères d'effets néfastes. Des différences dans le type d'habitat ainsi que dans la biodisponibilité du zinc présent dans ces sédiments peuvent expliquer ces variations.

Les résultats de l'échantillonnage supplémentaire effectué en 1991 à six sites-clé à proximité de Mattabi (éventail des concentrations de zinc dans les sédiments: 92-46,000 µg/g), a montré une réponse plus claire des communautés benthiques (i.i. diminution du nombre de taxons en fonction de l'augmentation des concentrations de zinc). Les résultats des tests de toxicité des sédiments effectués sur des échantillons provenant de ces sites n'ont démontré aucune toxicité avec *Hyalella azteca*. Quant aux résultats des tests utilisant *Chironomum* *tentans*, ils n'ont montré aucune différence significative avec le test-contrôle, et ce à des concentrations de zinc dans les sédiments allant jusqu'à 6000 µg/g. Par contre, une augmentation significative de la mortalité a été observée lorsque les concentrations de zinc étaient égales ou supérieures à 30,000 µg/g. Compte tenu de l'importance de cette base de données, il a été possible de calculer des niveaux d'effets mineurs et sévères spécifiques à ce site, selon la même méthode de concentration du niveau de dépistage (utilisée par le MEO). Les valeurs obtenues étaient généralement d'un ordre de grandeur plus élevées que les critères de qualité des sédiments du MEO.

Les résultats provenant de plans d'eau situés à proximité de Geco ont montré des tendances similaires à celles décrites près de Mattabi, tant au niveau des communautés benthiques ou des tests de toxicité des sédiments (taux de mortalité comparable au test-contrôle) à des concentrations dans les sédiments égales ou inférieures à 7000 µg/g Zn.

Les déterminations de concentrations de sulfures volatiles à l'acide (AVS) et des métaux extraits simultanément (SEM) (effectués sur une dizaine d'échantillons provenant des deux sites) n'a pas permis de prédire la toxicité de ces sédiments (selon le modèle SEM/AVS > 1 = effets toxiques). Par contre, lorsque les concentrations de zinc dans l'eau interstitielle des sédiments de surface dépassaient le critère de qualité d'eau, des effets sur la faune benthique étaient observés. La présence des métaux (en particulier le zinc) sous une forme relativement peu mobile (oxydes Fe/Mn, complexes organiques) pourrait expliquer les effets moins importants sur les organismes benthiques que ceux prédits par les critères de qualité des sédiments. Bien que les critères de qualité des sédiments actuellement disponibles permettent une évaluation des effets spécifiques à un site donné (tel qu'effectué à Mattabi et Geco) lorsque les résultats dépassent ces critères, de futurs critères génériques qui considéreraient d'autres facteurs régissant la disponibilité de ces métaux pourraient s'avérer plus adéquats. Cependant, les outils permettant de quantifier ces facteurs devront être identifiés et validés avant d'être appliqués à de tels critères d'évaluation de la qualité des sédiments.

DÉTERMINATION DU TAUX D'ADDUITS À L'ADN DANS LE FOIE DU MEUNIER NOIR (*Catostomus commerconi*) ET DE LA CARPE (*Cyprinus carpio*) COMME BIOMARQUEUR DE LA CONTAMINATION DU MILIEU AQUATIQUE. El. Adlouni, C., Tremblay, J., Walsh, P., Lagueux, J. et Poirier, G.G. Unité d'Environnement et Santé, Centre de recherche du CHUL, Sainte-Foy, Québec.

Introduction

La plupart des substances mutagènes ou cancérigènes forment des liaisons covalentes avec le DNA, le RNA ou les protéines, soit directement, soit après leur métabolisation en métabolites électrophiles par certains systèmes enzymatiques tels les MFO ("mixed function oxidase"). La formation d'adduits sur l'ADN est particulièrement intéressante parce que ce type de lésion constitue une étape importante dans le phénomène d'initiation d'un cancer.

Les HAP sont métabolisés par l'organisme en métabolites électrophiles qui forment des adduits sur l'ADN. La cancérogénicité de nombreux HAP est fonction de leur potentiel de formation d'adduits (1,2). Des études ont démontré que suite à une exposition prolongée à des substances toxiques, certains adduits persistent et ne sont pas réparés par les systèmes enzymatiques de réparation. Cette propriété rend possible l'utilisation de la mesure des adduits à l'ADN comme biomarqueur de l'exposition à des substances génotoxiques.

Pour mesurer le taux d'adduits à l'ADN d'un organisme, la méthode analytique doit répondre à deux conditions de base: i) être très sensible puisque le niveau d'adduits est souvent très faible (de l'ordre de un adduit par 10^6 à 10^9 nucléotides), ii) ne pas être spécifique à un xénobiotique en particulier et être applicable à toutes les substances chimiques cancérigènes. Ces critères sont satisfaits par la méthode de post-marquage au 32 p.

Échantillonnage

Dans ce travail, nous avons voulu évaluer l'utilisation de la mesure des adduits à l'ADN comme biomarqueur de la contamination du milieu aquatique. Les niveaux d'adduits ont été mesurés dans le foie de meuniers noirs (*Catostomus commersoni*) et des carpes (*Cyprinus carpio*) pêchés dans quatre sites dont on connaît le niveau de contamination en HAP des sédiments.

Des meuniers noirs ont été pêchés à deux sites situés en amont et en aval de l'embouchure de la rivière Saint-Louis dans le fleuve Saint-Laurent (lac Saint-Louis). Le site amont est plus directement touché par les effluents du complexe industriel de Beauharnois; des teneurs d'environ 70 ppm en HAP totaux y ont été mesurés dans les sédiments (Laliberté 1991a). Les niveaux sont plus faibles au site aval (4 ppm). Étant donné que l'ensemble du secteur est fortement contaminé et que les poissons ne sont pas limités par aucune barrière physique, l'exposition des poissons aux HAP est considérée élevée aux deux sites.

Des meuniers noirs et des carpes ont été pêchés en amont et en aval d'un barrage à Windsor (Québec). Le site aval est soumis aux effluents d'une usine de pâtes et papiers. Les poissons du site aval montrent aussi une plus forte contamination en dioxine et furanne (Laliberté 1991b).

Principe de la méthode de mesure des adduits à l'ADN par post-marquage

a) Extraction de l'ADN

Les tissus sont tout d'abord broyés et soumis à une digestion avec la protéinase K. Par la suite, les acides nucléiques sont extraits au phénol et chloroforme et précipités dans l'éthanol 95% à -20°C. L'ARN est par la suite éliminé par traitement avec des RNAses.

b) Hydrolyse de l'ADN et marquage des adduits

Une quantité de 10 µg d'ADN des échantillons est prélevée et hydrolysée enzymatiquement avec la nucléase micrococcale (MN) et la phosphodiestérase de rate (SPDE). La réaction hydrolyse l'ADN en 3'-phosphodésoxynucléotides normaux (3'p-dN) et adduits (3'p-dN-X). Un adduit est formé par la liaison covalente d'un xénobiotique ou d'un de ses métabolites à un nucléotide.

ADN + MN + SPDE 3'p-dN + 3'p-dN-X

L'ensemble des nucléotides normaux et des adduits est ensuite soumis à l'action de la nucléase P1 qui enlève le phosphate en position 3‡ sur les nucléotides. Du fait de l'encombrement stérique engendré par le xénobiotique, la nucléase P1 ne déphosphoryle que les nucléotides normaux.

3'p-dN + 3'p-dN-X + P1 dN + pi + 3'p-dN-X

On procède ensuite au marquage enzymatique à l'aide de la T4 polynucléotide kinase (T4-PKN) en présence de (^{32}P) ATP. La T4-PKN ajoute un phosphate en 5' aux 3'nucléotides. Du fait de la présence d'un phosphate à leur extrémité 3', seuls les adduits seront donc phosphorylés en 5'.

3'p-dN-X (^{32}P)ATP + T4-PKN 5'^{32}p-dN-X-3'p + ADP

c) Séparation des adduits par chromatographie sur couche mince

Les milieux réactionnels sont déposés à 2 cm du bas d'une plaque de polyéthylèneimine (PEI)-cellulose qui l'on fait migrer (1ère migration) dans un solvant polaire qui entraîne les molécules hydrophiles (nucléotiques normaux, ^{32}P) alors que les molécules hydrophobes (adduits) restent à l'origine. Les adduits sont ensuite transférés sur de nouvelles plaques de PEI-cellulose et celles-ci sont développées avec des solvants de moindre polarité en deux dimensions (2ième et 3ième migrations) pour augmenter leur séparation. Les solvants utilisés dépendant de la nature des adduits que l'on cherche à détecter.

Les chromatogrammes sont par la suite autoradiographiés et les taches sont excisées et comptées en Cerenkov au compteur à scintillation. Les résultats sont exprimés en nombre d'adduits par 10^9 nucléotides normaux sachant que 1 µg d'ADN correspond à 3,03 nmoles de nucléotides.

Résultats et discussion

a) Profils chromatographiques et niveau total d'adduits

1. On a retrouvé 10 taches identiques sur les chromatogrammes des meuniers noirs du lac Saint-Louis et de la rivière St-François. Ces taches correspondent à des adduits qui sont soit endogènes, étant donné qu'on les retrouve dans tous les cas pour des concentrations variables

de polluants, soit exogènes, c'est-à-dire qu'ils peuvent provenir de contaminants non-identifiés présents en quantité similaire dans les quatre sites.

2. Les meuniers noirs du lac St-Louis montrent 7 taches (ou adduits) supplémentaires que l'on attribue à la contamination plus importante de ces sites en HAP.

3. Les deux sites de la rivière St-François montrent des profils chromatographiques similaires.

4. On note une différence importante entre les profils chromatographiques des meuniers noirs et des carpes. Les chromatogrammes des meuniers noirs de la rivière St-François montrent une distribution alignée de 10 taches tandis que ceux de la carpe montrent un groupe de 6 à 7 taches au centre du chromatogramme. Les variations spécifiques peuvent provenir de différences dans l'exposition aux contaminants (ex.: régime alimentaire) ou dans leur métabolisme.

B) Les adduits à l'ADN comme biomarqueur d'exposition à des contaminants génotoxiques

5. Nous avons réussi à détecter des niveaux de 4 adduits par 10^9 nucléotides, ce qui démontre la grande sensibilité de la méthode de mesure des adduits par post-marquage au ^{32}P.

6. La mesure des adduits à l'ADN du foie de poisson confirme que les effluents du complexe industriel de Beauharnois et particulier ceux de l'aluminerie entraîne une plus grande exposition des poissons à des substances génotoxiques que l'on croit être des HAP.

7. Par ailleurs, la mesure des adduits n'a pas permis de mettre en évidence une exposition additionnelle à des substances génotoxiques chez les poissons de la rivière St-François qui sont soumis aux effluents d'une usine de pâtes et papiers.

C) Limites et amélioration possibles à la méthode.

8. Le nombre d'adduits peut être sous-estimé étant donné que:

- certains adduits moins résistants à la nucléase P1 peuvent être déphosphorylés et peuvent ne pas être marqués par la kinase;
- la perte de certains adduits hydrophiles n'est pas exclue.

9. L'utilisation simultanée d'autres méthodes d'enrichissement (ex.: extraction au butanol) ou d'autres solvants pourraient augmenter la récupération des adduits et donner plus d'information sur leur structure chimique.

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Remerciements

Les auteurs désirent remercier pour leur participation à ce projet MM. Denis Laliberté (Environnement Québec) et Jacques Bureau (Environnement Canada).

Ce travail a été rendu possible grâce à une subvention du Conseil national de la recherche en science et génie du Canada.

Site	Espèces	Sexe	Poids (g)	Longueur (cm)	Adduits (/10^9 nucl)
Lac St-Louis:					
Site A	mn	M	1140	44.3	132
	mn	F	758	40.4	120
	mn	M	894	42.2	90
Site B	mn	M	1431	48	137
	mn	F	1030	41.7	123
	mn	M	1337	47.8	142
Rivière St-François:					
Site C	mn	M	1010	41	72
	mn	M	1060	42	70
	mn	F	1250	47	80
	c	M	1180	46.9	100
	c	M	1400	52	121
	c	F	1740	55.8	90
Site D	ws	M	1120	44	85
	ws	M	910	42.9	63
	ws	F	1020	43.5	74
	c	M	1210	49	96
	c	F	1590	53.7	83
	c	F	1340	49	73

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mn: meunier noir
c: carpe
nucl: nucléotides

LA MACROAUTORADIOGRAPHIE: UN OUTIL POUR LES ÉTUDES DE TOXICOLOGIE ENVIRONNEMENTALE. WHOLE BODY AUTORADIOGRAPHY: A TOOL FOR ENVIRONMENTAL TOXICOLOGY STUDIES.

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RÉSUMÉ

La macroautoradiographie donne la possibilité de visualiser rapidement et en détail la distribution d'un composé chimique marquée avec in isotope radioactif dans tous les tissus d'un organisme vivant. Cette technique consiste à faire des cryosections fines (20-60 µm) d'un animal entier (poisson, souris, étoile le mer) auquel a été administré (injection intraveineuse ou péritonéale, contact direct, nourriture) un composé radioactif. Ces sections sont ensuite mises en contact avec un film radiographique pour une période de quelques semaines à quelques mois. L'image ainsi obtenue permet de localiser précisément le composé chimique administré à l'animal. La résolution de l'image est telle que des détails de moins de 100 µm peuvent être visibles.

Mise au point en Suède au début des années 50, la macroautoradiographie a surtout été utilisée en recherche médicale et pharmaceutique. L'application de cette technique lors de nos travaux sur l'accumulation d'ions métalliques et de composés organométalliques chez la truite et l'étoile de mer a démontrée que la macroautoradiographie peut aussi être très utile en toxicologie environnementale, bien qu'elle soit très peu utilisée à cette fin. La macroautoradiographie facilite l'orientation des recherches en permettant d'identifier rapidement les sites d'accumulation d'intérêt. C'est une technique simple et rapide qui peut donner de nombreuses informations très difficiles à obtenir autrement. C'est une technique de choix à considérer pour l'évaluation environnementale des nouveaux composés chimiques avant leur lancement sur le marché... ou dans l'environnement.

ABSTRACT

Whole body autoradiography allows a fast and easy visualization of the distribution of radiolabelled compounds in organs and tissues of a whole animal. The technique is based on thin cryosectionning (20-60 µm) of a whole animal (fish, mouse, starfish) to which a radiolabelled compound has been previously administered (injection, direct contact, food). These sections are then applied to radiography film for a period of a few weeks to a few months. Images obtained this way show a high resolution (details smaller than 100 µm are visible) and allow a very precise localization of chemicals administered.

Whole body autoradiography has been set at the beginning of the 50's, in Sweden, and is commonly used in medical and pharmaceutical research. Its application in the course of our studies on the uptake of metallic ions and organometallic compounds in the brown trout and the starfish showed its usefulness for environmental toxicology studies, although it is not currently used at present in this research field. Whole body autoradiography facilitates orientation of research by allowing easy identification of organs and tissues of interest. This fast and easy technique can give information on the tissue distribution of a given compound very difficult to obtain otherwise. This is a valuable tool for the environmental evaluation of new chemicals before their release on the market ... or in the environment.

INTRODUCTION

L'autoradiographie utilise la propriété qu'ont les particules ou les rayonnements émis par les éléments radioactifs de noircir les films photographiques, comme le fait la lumière visible. L'utilisation de l'autoradiographie s'est surtout répandue après la deuxième guerre mondiale. La fission nucléaire contrôlée permettant de produire artificiellement toute une variété d'éléments radioactifs, il devint possible d'étudier la distribution d'une molécule quelque marquée avec un élément radioactif. L'autoradiographie devint un outil courant en recherche médical et pharmaceutique, puisqu'elle permettrait d'étudier la distribution de composés chimiques d'intérêt, comme les nouveaux médicaments. Deux problèmes techniques majeurs existaient cependant. La plupart des produits étudiés chez l'animal étant solubles dans l'eau ou dans les liquides utilisés lors des procédures de fixation histologique (fixation, déshydratation, décalcification, etc...), ils pouvaient être extraits du tissu ou changer de localisation dans le tissu. De plus, l'investigation était le plus souvent limitée à certains tissus pré-sélectionnés. Il est toujours possible d'examiner un à un tous les tissus d'un organisme vivant, mais cela est pratiquement difficile et coûteux.

Mise au point en Suède au début des années 50 (Ullberg, 1954, 1977), la macroautoradiographie est basée sur l'obtention de cryosections d'un animal entier. La congélation permet de préserver l'intégrité de la distribution du composé dans les tissus et de visualiser d'un coup d'oeil la distribution *in situ* du composé étudié dans tous les tissus et fluides de l'organisme (glandes endocrines, les diverses parties de l'oeil, de l'adent, du système digestif, du système nerveux, des os, etc...). Jusqu'à présent, la macroautoradiographie a surtout été utilisée en recherche médicale et pharmaceutique. Cet article a pour but de décrire brièvement cette technique et d'en démontrer l'utilité pour les études de toxicologie environnementale.

MATÉRIEL ET MÉTHODES

À peu près tous les isotopes radioactifs sont utilisables en macroautoradiographie, que ce soit un émetteur de particules β à faible énergie comme ^{3}H, un émetteur d'électrons Auger (^{125}I, ^{113}Sn, ^{54}Mn), un émetteur de particules β plus énergétiques (^{14}C, ^{35}S, ^{203}Hg) ou un émetteur de particules α (^{238}Pu).

Le composé marqué à l'étude est administré à un animal (poisson, souris, rat, singe, étoile de mer, caille; jusqu'à 45 cm de longueur et 15 cm de largeur), qui sera sacrifié après un certain temps. L'animal est inclus dans un gel de carboxyméthylcellulose et congelé rapidement dans l'hexane refroidit à -78°C avec de la glace sèche. Des sections (20 μm d'épaisseur en général) sont faites à -20°C à l'aide d'un cryomicrotome spécialement conçu à cet effet (LKB 2250 PMV, PMV Co., Stockholm, Suède). Lorsqu'un tissu ou un organe d'intérêt est visible à la surface du bloc, un morceau de ruban adhésif (3M, type 810) y est appliqué de sorte que la section y adhère une fois coupée. En faisant plusieurs sections à divers niveaux, on peut ainsi échantillonner tous les tissus de l'animal. Les sections sont ensuite lyophilisées à -20°C pour finalement être appliquées sur un film radiographique. La plupart des films radiographiques courant, comme le AGFA Structurix, sont utilisables avec les émetteurs de particules α et β à haute énergie. Un film spécial (Hyperfilm ^{3}H, Amersham) est utilisé avec les émetteurs d'électrons Auger et de particules β à faible énergie. L'exposition se fait à -20°C et dure de quelques semaines à quelques mois. Après développement, l'image obtenue peut être comparée à la section pour localiser le composé marqué.

QUELQUES RÉSULTATS

Les travaux au cours desquels nous avons utilisé la macroautoradiographie ont porté sur la bioconcentration de métaux en trace [Mn(II), Hg (II), CH^3Hg(II), Cd(II), Ni(II), Pb(II)] chez la truite brune *Salmo trutta* (Gottofrey et Tjälve, 1991; Rouleau *et al*, 1993a; Tjälve et Gottofrey, 1991; Tkälve *et al*, 1986, 1988), les effets d'agents chélatants sur la bioconcentration des métaux en trace chez la truite brune (Borg *et al*, 1988; Gottofrey, 1991), et la bioconcentration (Rouleau *et al*, 1993c) et le transfert trophique (Rouleau, Pelletier et Tjälve, en préparation) de Hg(II), CH_3Hg(II), Sn(IV) et But_3Sn(IV) chez l'étoile de mer *Leptasterias polaris*. Les exemples suivants permettront de démontrer la grande utilité que peut avoir la macroautoradiographie pour les études de toxicologie environnementale.

Lors de nos travaux sur la bioconcentration du manganèse chez la truite brune (Rouleau *et al*, 1993a), les autoradiogrammes ont mis en évidence l'accumulation de manganèse dans le cerveau via le système olfactif (rosette olfactive → nerf olfactif → bulbe olfactif → télencéphale; Fig. 1). La macroautoradiographie a ici permis d'observer l'accumulation de manganèse dans un tissu d'accès très difficile; chez des truites de 6 à 9 cm de longueur, comme celles utilisées dans nos expériences, le nerf olfactif mesure quelques millimètres de longueur, de 200 à 300 μm de diamètre et il est inclus dans la matrice osseuse du crâne. La neurotoxicité de doses élevées de manganèses est connu (Mena *et al*, 1967), et cette voie d'accès directe au cerveau chez le poisson pourrait être un facteur toxicologique déterminant chez les populations de poissons exposés à de hautes concentrations de manganèse dissous (Rouleau *et al*, 1993a).

Il est connu que les agents pouvant complexer les ions métalliques dissous peuvent en modifier la vitesse d'accumulation dans les organismes aquatiques. Il est bien sûr possible de déterminer quantitativement ces différences (dissection et dosage des tissus prélevés). La macroautoradiographie permet de visualiser ces changements, parfois saisissants, et de donner des informations sur les tissus trop petits, trop fragiles ou trop diffus pour être disséqués. La figure 2 montre des autoradiogramme provenant de truites brunes exposées au cadmium seul, ou au cadmium et au diéthyldithiocarbamate de sodium (Gottofrey *et al*, 1988a). La différence de distribution est tout à fait évidente. De plus, la macroautoradiographie permet d'obtenir des renseignements supplémentaires sur les effets de cet agent chélatant, comme l'accumulation de cadmium dans le coeur, le sang, la peau et les muqueuses gastrique et orale.

Durant nos travaux sur le transfert trophique de composés organométalliques chez l'étoile de mer *leptasterias polaris*, nous avons utilisé des modèles compartimentaux pour quantifier les cinétiques observées (Rouleau, Pelletier et Tjälve, en préparation). Un des postulats de base de tels modèles est qu'un composé entrant dans un compartiment est instantanément distribué à tous les tissus constituant le compartiment (Barron *et al*, 1990). Le processus digestif s'étale sur 12 à 24 heures chez *L. polaris*. Nous avons voulu vérifier si il n'y avait pas création d'un gradient de concentration durant la digestion dans certains organes, comme les caeca pyloriques, ce qui aurait eu pour effet d'invalider le postulat de la distribution instantanée. Des autoradiogrammes (Fig. 3) d'étoiles de mer échantillonnées 6, 16 et 48 heures après l'ingestion de nourriture contaminée n'ont permis d'observer aucun gradient de concentration dans les caeca pyloriques, validant ainsi le postulat d'une distribution très rapide, si non instantanée, des produits marqués y pénétrant.

CONCLUSION

Jusqu'à présent, la macroautoradiographie a été très peu utilisée en toxicologique environnementale, bien qu'elle puisse rendre de grands services. Si cette technique a un défaut, c'est l'importante dépense d'immobilisation nécessaire pour l'acquisition du matériel de base, le prix du cryomicrotome à lui seul frôlant les 200 000 dollars. Cependant, la macroautoradiographie est simple, rapide, relativement peu coûteuse à l'usage, et elle donne des informations qui peuvent être très difficiles à obtenir autrement. De plus, elle facilite l'orientation des recherches en permettant d'identifier rapidement les sites d'accumulation d'intérêt. Ceci en fait une technique de choix à considérer pour l'étude de la toxicologie environnementale des polluants déjà identifiés et pour l'évaluation environnementale des nouveaux composés chimiques.

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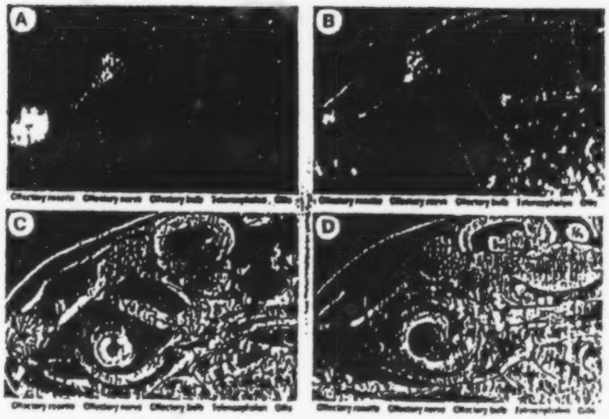
A
B
C
D
Olfactory rosette
Olfactory nerve
Olfactory bulb
Telencephalon
Gills

Fig. 1: Macroautoradiogramme d'une truite brune (Salmo trutta) exposée pendant 3 semaines (A) et 6 semaines (B) à 0,1 µg ^{54}Mn(II)·L^{-1}. (C) et (D) sont les sections correspondants à (A) et (B) respectivement.

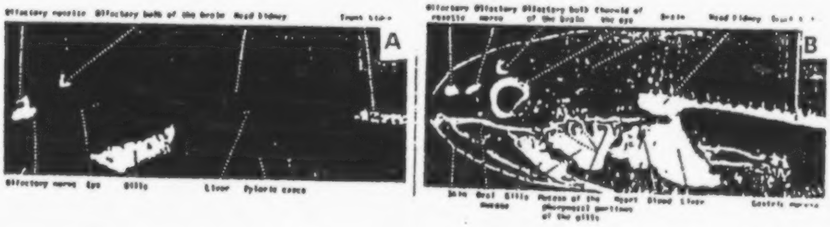
Olfactory rosette
Olfactory bulb of the brain
Head kidney
Trunk kidney
A
Olfactory nerve
Eye
Gills
Liver
Pyloric caeca
Olfactory rosette
Olfactory nerve
Olfactory bulb of the brain
Choroid of the eye
Brain
Head kidney
B
Skin
Oral mucosa
Gills
Mucosa of the pharyngeal portions of the gills
Heart
Blood
Liver
Gastric mucosa

Fig 2.: Effets du diéthyldithiocarbamate de sodium (SEC) sur la bioconcentration du cadmium (0,1 µg ^{109}Cd(II)·L^{-1}) chez la truite brune. (A) Cd seul, (B) Cd + SEC (Gottofrey et al. 1988a).

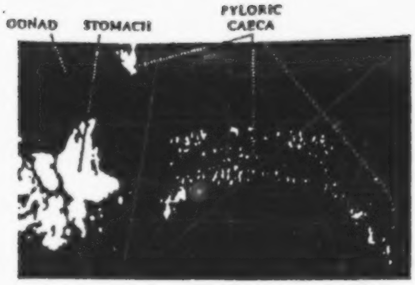
GONAD
STOMACH
PYLORIC CAECA

Fig. 3: Autoradiogramme d'une étoile de mer (Leptasterias polaris) 6 heures après l'injection de nourriture contaminée avec 0,3 $\mu g \cdot g^{-1}$ de ^{113}Sn(IV).

DEGRADATION AND TOXICITY OF BUTYLTIN COMPOUNDS IN STARFISH

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The starfish *Leptasterias polaris*, an important predator in the benthic community of the St.Lawrence Estuary (eastern Canada), was used in assessing the degradation potential and toxicological implications of tributyltin (TBT) ingested with food. TBT was chosen as a representative of the organotins, a group of wide-spread chemicals used as biocides and plastic stabilizers. To our knowledge, attention has not been given to a detailed evaluation of organotin fate from prey to predator with reference to potential reproductive impairment. This work combines an accurate determination of TBT budget through trophic transfer, a speciation study of the contaminant in the digestive and reproductive systems, and an investigation of the physiological/morphological effects on gonadal development.

Male and female starfish were fed daily with TBT contaminated mussels for a 53-day period. the exact amount of ingested tissue was obtained by weighting the whole mussel served to a starfish and subtracting mass of recovered empty shell. A homogenate of contaminated mussels was analyzed for TBT content, which permitted a daily determination of TBT burden given to each starfish (4.80 ± 0.50 μg TBT/ g wet mussel mass). The target organs (gonads and pyloric caeca) were sampled at regular intervals. Determination of TBT and degradation products (dibytyltin and monobutyltin) was performed by coupled gas chromatography-mass spectrometry (GC-MSD) with a detection limit of 1 ng for each butyltin species which corresponds to a practical detection limit of 2.5 mg/ g wet mass for the tissue analyzed. The determinations of total tin were performed using coupled hydride formation system (MHS-10 Perkin-Elmer) and atomic adsorption spectroscopy (AAS). After performing a standard embedding procedure, 8-9 μm slices of the gonads were prepared and subjected to periodic acid-Shiff (PAS) staining procedures which give a pink coloration to nutritive reserves (glycogen and other polysaccharides).

Sequential debutylation occurred in the starfish within 2 weeks, first yielding dibutyltin (DBT), which seemed to accumulate more readily in the ceaca (up to 1.10 μg/ g), then monobutyltin (MBT), which appeared only in few individuals at low concentrations (below 0.35 μg/g). Males and females were characterized by similar time dependent accumulation profiles for debutylation residues in both cases, DBT was the major accumulated product and accounted for 67 ± 9% (males) and 70 ± 12% (females) of the total butyltins present in organs after 53 days. TBT content, combined for pyloric caeca and gonads, remained essentially constant at 3.19 ± 0.83 μg for males and 3.48 ± 1.21 μg for females. For both sexes, the tin burden was far greater in the pyloric caeca, and only 5.0 ± 1.9% of the total tin was recovered in the gonads. Although the total amount of TBT ingested from food continuously increased throughout the experiment (mean of 657 μg for a starfish fed over 53 d), the total amount of tin (Sn) traced in the analyzed starfish tissues remained below 80 μg, suggesting an excretion system and/or a storage of tin in an undetectable form. The molecular form of tin recovered by tissue digestion and analysis by AAS is not well established. Inorganic tin (IV), resulting from complete debutylation process, is likely to be present but the large amount of total tin recovered in starfish after 6-8 weeks is suspected to be partly degraded butylin compounds such as β-(hydroxybutyl)dibutyltin (Lee et al. 1989) that are not detected by the GC-MSD method.

Although all species of the contaminant remained barely detectable in the gonads, micrometric measurements in histological preparations revealed subtle physiological anomalies in gonadal epithelia and oocytes (PAS-positive). In comparing with structures of control starfish, no significant differences were noted for data from the first 3 days ($p=0.177$ for males and $p=0.279$ for females). However by day 39, contaminated individuals of either sex were characterized by significantly thinner gonadal epithelia ($p<0.001$). A similar pattern was observed in females for oocyte diameters, both previtellogenic (PO) and in final maturation (OM), which were similar to those of controls (PO: $p=0.234$, OM: $p=0.195$) at the beginning of the experiment and significantly smaller after day 39 ($p<0.001$).

The most probable degradation pathway of TBT in starfish would be through a cytochrome P-450 dependant enzyme, well characterized in rat liver, where TBT undergoes hydroxylation yielding (hydroxybutyl)dibutyltin derivatived. Microsomal fractions from caeca of the starfish *Asterias subens* possess mixed function oxygenase activity, including cytochrome P-450 (Den Bestern et al. 1990, 1992). What can be inferred from the histological results is the probable loss of reserves reflected by the thinning of gonadal epithelia as well as the reduced ability to sustain oocyte development for the contaminated starfish. Usually, during the summer months corresponding to our experimental period, starfish *Leptasterias polaris* undergo early gametogenesis of gamete synthesis (Boivin et al. 1986). Since the development in *L. polaris* is lecithotrophic (5-6 mo), it relies on abundant yolk reserves stored in the eggs which is why the oocyte population recruited for spawning is primarily that having attained a size of 600-800 μm at maturity. As for contaminated individuals, the remarkable thinning of epithelia indicates that the nutrients absorbed from diet seemed not accessible to the gonads and that previously stored reserves were utilized, allowing only a limited oocyte development. The outcome for contaminated starfish is a dominant population of small maturing oocytes which could be detrimental to subsequent development. Therefore, TBT or this metabolites interferes with gonadal development, whether through hormonal regulation process by cytochrome P-450 enzymes or inter-membrane nutrient transport to gonads. The first hypothesis involves the enzymatic pathway of the P-450 and its essential role in hormone synthesis (Hall 1986) which could be affected by the binding of the contaminant to enzyme sites, resulting in impaired activity. This should simultaneously affect the metabolism of TBT, as it is completed through the same pathway. In fact, our results indicate a less efficient degradation after day 25 with greater accumulation of DBT and increased BT/total Sn proportion in organs.

These results show sea stars and possibly other echinoderms, avoid immediate accumulation of TBT by dealkylating the toxicant to less harmful metabolites. However, this natural defense process was only partly effective and subtle effects on reproductive organs were induced by disturbing reserve acquisition in growing gametes. The starfish, a long-living predator is proposed as an excellent organism for the study of trophic transfer and related toxic effects of organotins and other marine pollutants in coastal and estuarine areas. Although butyltin contamination from antifouling paints is under strict legislative control in western countries, the annual world production of organotin compounds reached 50 000 tonnes in 1992. Further, restrictions of TBT uses have not appreciably diminished the consumption of triorganotin biocides world-wide and important TBT concentrations can still be found in some areas (Espourteille et al. 1993), probably from accumulation in sediment (Fent & Hunn 1991). Therefore, the threat of organotins to aquatic organisms will continue for years and studies of chronic and low level toxic effects of alkyl- and aryl-tins are urgently needed.

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THE METHYL-MERCURY IN POREWATERS OF ANOXIC MARINE SEDIMENT FROM THE SAGUENAY FJORD.

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ABSTRACT

Methyl-mercury, $CH_3Hg(II)$, the main form of mercury in fish, is more toxic than inorganic mercury. Until now, it was assumed that the methyl-mercury production resulted from strong bacterial activity in contaminated and organic-rich surface sediments. The distribution of $CH_3Hg(II)$ in sediment porewaters, however, has rarely, if ever, been measured directly. Consequently, the behaviour of $CH_3Hg(II)$ in marine sediments and its flux at the sediment-water interface remain unknown.

Dissolved $CH_3Hg(II)$ was analyzed in porewaters extracted from Saguenay Fjord sediments sampled at three different stations in the landward basin. These sediments are characterized by high mercury concentrations due to industrial loading from a chlor-alkali plant and a very thin oxidized layer (<1 cm) below which strongly reductive conditions were detected. $CH_3Hg(II)$ concentrations in porewaters were relatively high below the redox interface and reached up to 10 ng Hg L^{-1} (50 pM). Profiles also indicated that, despite a high concentration gradient, little $CH_3Hg(II)$ escapes to the overlying water column because the oxidized sediments at the interface seem act as an efficient boundary to the release of methylmercury into the water column. In the oxic zone, $CH_3Hg(II)$ may be sorbed by fresh organic matter and iron oxyhydroxides or subjected to the demethylation processes.

INTRODUCTION

Sediments play an important role for the distribution of different compounds in aquatic systems as a sink or a source. The origin of methylmercury, available to the bioaccumulation in aquatic organisms, is still unclear. To evaluate the potential hazard of mercury in an aquatic environment, it is now generally recognized that the most important factor to be considered is its methylmercury ($CH_3Hg(II)$) content rather than the amount of total mercury. It is generally believed that most $CH_3Hg(II)$ in waters is generated in contaminated organic-rich surface sediments (Furutani and Rudd, 1980; Parks et al., 1989). The fate of $CH_3Hg(II)$ in marine sediments is not well understood yet, but its biogeochemistry is important to evaluate the possible $CH_3Hg(II)$ source towards the water column.

The methylation of mercury in natural environment can occur biologically by the action of bacteria or, a biologically, by the reaction of inorganic Hg(II) with some methyl donors such as CH_3I (Berman and Bartha, 1986). The synthesis of $CH_3Hg(II)$ occurs primarily, but not exclusively, in anoxic aquatic sediments (Jensen and Jernelov, 1969; Parks et al., 1989; Matilainen et al., 1991). Sulfate-reducing bacteria are principal methylators of Hg in anoxic estuarine sediments (Campeau and Bartha, 1985). The amount of available inorganic Hg and the microbial activity control the methylation of Hg (Jackson, 1988). Sulphides produced in anoxic sediments decrease the availability of inorganic Hg by strongly bounding Hg (HgS) (Bartlett and Craig, 1981). To date, measurements of $CH_3Hg(II)$ have been performed on total sediments (e.g., Tompson et a., 1980; Barlett and Craig, 1981; Craig and Moreton, 1986), and results of dissolved $CH_3Hg(II)$ in porewaters have not yet been published.

METHODS

Sediment cores were collected at three stations located along the main axis of the Saguanay Fjord, Canada (Fig. 1); near the head of the fjord (SAG-6; 100 m deep), at the junction of the Baie des Ha!Ha! (SAG-15; 180 m deep), and in the deepest portion of the landward basin (SAG-30; 265 m deep). A fourth sampling station was situated offshore from Rimouski (Quebec) in the St.Lawrence Estuary (CL-1; 335 m deep).

Undisturbed sediments were sampled with a box corer (0.12 m^3) and subsampled at various depth intervals in a nitrogen-filled glove-box. Porewater extraction was performed using "Reeburgh type squeezers". the porewater samples were stored without any chemical preservative added (Bloom, 1989) and kept frozen until analysis. Samples for the determination of total dissolved Hg were acidified with ultra-pure nitric acid to make a 1% HNO_3 solution. All handings were performed using rigorous trace-metal-clean protocols (Gill and Fitzgerald, 1987).

The analytical method of methylmercury in porewaters was adapted from Bloom and Fitzgerald's technique (Bloom, 1989; Bloom and Fitzgerald, 1988). $CH_3Hg(II)$ was analyzed after derivitization using sodium tetrathylborate where volatile alkylmercury compounds are trapped, separated using gas chromatography (GC), and measured by cold vapour atomic fluorescence. Prior to ethylation, a pre-extraction using a solvent must be performed to separate $CH_3Hg(II)$ from chloride ions and partially from dissolved organic matter which could interfere with the ethylation reaction. $CH_3Hg(II)$ in porewater samples was extracted with methylene chloride after treatment with copper sulphate and acidic solution of potassium bromide (Avery, 1989). $CH_3Hg(II)$ was then back extracted into buffered water (pH 4.9) following solvent evaporation (Bloom, 1989). The cryogenic GC system is replaced by an isothermic GC separation (T=90°C) for the chromatographic separation procedure. The use of a Tenax® trap rather than a graphitized carbon column allowed a rapid desorption of trapped Hg species at lower temperature (275°C) and a direct coupling to GC (Van Tra et al., submitted). The limit detection was 0.3 ng L^{-1} as Hg. Recovery of 0.7-2.1 ng as Hg of methyl mercuric chloride spiked in porewater samples at the start of the procedure was 92 ± 5%.

Total dissolved Hg analysis, including organic and inorganic species, was performed by gold amalgamation preconcentration in conjunction with cold vapour fluorescence atomic (CVFA) detection (Gill and Fitzgerald, 1987) after cold oxidation by BrCl or organic compounds (Bloom and Creceliu, 1983). Mercury in the solid phase was measured following the method of Toth and Ingle (1977). Dissolved Fe in the porewaters was determined on acidified samples by flame AAS. Total carbon was determined on homogenized freeze-dried sediments using a Carbo-Erba elemental analyzer.

RESULTS AND DISCUSSION

Figure 2 shows the depth distributions of dissolved $CH_3Hg(II)$ in porewaters. At all stations, minimum concentrations (<0.3 ng L^{-1} as Hg) appeared in oxidized surface sediments. Relatively large amounts of dissolved $CH_3Hg(II)$ were measured (up to 10 ng L^{-1} as Hg) in the anoxic zone. In general, the dissolved $CH_3Hg(II)$ corresponds to 10-20% of the total dissolved Hg and seems not limited by the amount of total dissolved Hg in these sediments. Porewater Hg concentrations are relatively high (17-508 ng L^{-1}) but represent generally only about four magnitudes of order lower than total sediment Hg contents. Variations in dissolved iron profiles point out the surficial sediment as an oxic zone and indicate the Eh transition between oxic and anoxic zone by the dissolution of hydr(oxides) iron in reducing environments. Generally, the dissolved Hg contents increase near the sediment-water interface due to the release of trapped Hg onto hydr(oxides) iron as a probable consequence of the microbial degradation of organic matter.

In spite $CH_3Hg(II)$ concentrations in porewaters from other studies are not available for comparison purposes, the $CH_3Hg(II)$ concentration measured in Saguenay Fjord sediments can be interestingly compared to valued obtained in anoxic bottom lake waters where concentrations ranged also from 3-10 ng L^{-1} (Furutani and Rudd, 1980; Bloom, 1989; Bloom et al., 1991). However, concentrations in porewaters are weak when comparated to those measured by other workers in some whole sediments which range generally 0.2-9 ng g^{-1} as Hg dry weight (Thompson et al., Craig and Moreton, 1986).

Due to the large concentrations of dissolved Fe (>10 mg L^{-1}), the H_2S concentration is not expected to be high in porewater of Saguenay Fjord sediments. In spite of relatively high sulphate reduction rate values reported by Edenborn et al. (1987), concentrations of H_2S in porewaters are low (< 6 μm; Gagnon, unpublished data). There would be more bioavailable Hg due to the interaction of iron with sulphur which can cause a strong binding of Hg (Matilainen et al., 1991). $CH_3Hg(II)$ levels in estuarine and coastal sediments seem to be controlled more by the sufphide content and negative Eh than by factors such as total Hg levels or organic matter content in sediments (Craig and Moreton, 1983). High methylation rates in sulphide-rich sediments have been observed by Futurani and Rudd (1980) in fresh water sediments, though, which indicates that the sulphide was in the from of insoluble FeS. In landslide deposits resulting from a catastrophic landslide in 1971 in upstream, high amounts of $CH_3Hg(II)$ were measured (see SAG-6 and SAG-15). This environment, where H_2S concentration is low, seems to have favourable conditions for Hg methylation and/or preservation of $CH_3Hg(II)$.

In spite of high $CH_3Hg(II)$ contents in porewaters, the diffusion of methylmercury from sediment bulk to the water column seems to be weak. The concentration gradient at the sediment/water interface was very low. The demethylation microbial activity could be highly significant within oxic surface sediments. Furthermore, iron oxides may act as a sink for $CH_3Hg(II)$ at the sediment surface, thereby limiting the flux of mercury to the overlying bottom water. This interpretation is consistent with the existing results of diffusive fluxed of total dissolved Hg from sediments (Bothner et al., 1980; Gobeil and Cossa, in press), which demonstrates that only sediments having strongly reducing conditions in the surface layer can release detectable amounts of mercury to the water column. Therefore, in the Saguenay Fjord environment, little $CH_3Hg(II)$ from sediment could enrich the overlying oxic water column. Nevertheless, the burrow organisms could favour the transfer to water column by bio-irrigation (Boddington et al., 1979(but their action has not been determined in the present study.

CONCLUSION

Anoxic non-sulphidic sediments are greatly conductive to the formation of $CH_3Hg(II)$. Our results suggest that methylated Hg in anoxic sediments could not significantly diffuse through the oxidized sediment layer towards the overlying water but could enrich this sediment zone where $CH_3Hg(II)$ is likely adsorbed and/or dimethylated, and then associated with Fe-Mn oxides. So, we can expect that the Hg-bioaccumulation in fish comes from its food, such as benthic organisms, rather than its contact to the dissolved $CH_3Hg(II)$ in the oxic water column.

ACKNOWLEDGEMENTS

We wish to thank the Dr W.F. Fitzgerald's group at the University of Connecticut for teaching recent techniques on the mercury speciation in natural waters. This work was funded by the St.Lawrence Centre/NSERC and DFO/NSERC Joint Subention program grants. This publication is a contribution of the Oceanographic Centre of Rimouski (Quebec, Canada).

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APPLICATION OF SEDIMENT BIOASSAYS FOR THE ENVIRONMENTAL ASSESSMENT OF OCEAN DUMP SITES

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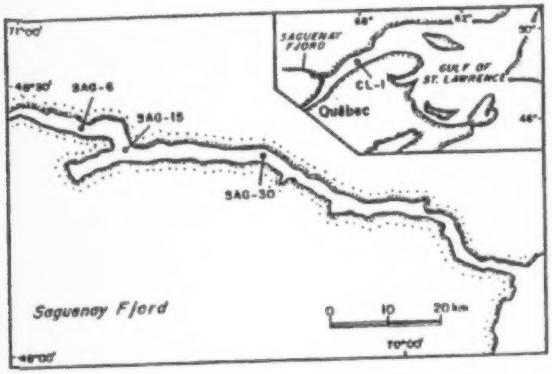
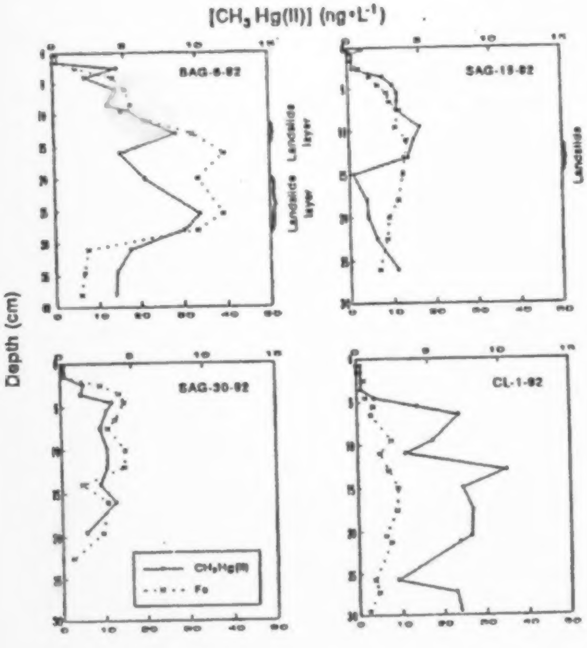

71°00'
48°30'
SAG-6
SAG-15
SAG-30
Saguenay Fjord
0
10
20 km
70°00'
48°00'
SAGUENAY FJORD
68°
62°
50°
CL-1
GULF OF ST. LAWRENCE
Québec
46°

Figure 1. Map of the Saguenay Fjord and St. Lawrence Estuary showing coring sites (SAG-6, SAG-15, SAG-30 and CL-1).



3. **Sea Urchin Fertilization Test (*L. pictus*):**

- sediments are toxic if fertilization is at lest 25 percent lower than control sample fertilization and statistically different.

4. **Bacterial exoenzyme test:**

- sediments are toxic if control site activity is reduced by 20 per cent or more of the normalized dilution value at a test sediment concentration of 25 per cent.

BIOCONCENTRATION FROM WATER AND BIOACCUMULATION FROM FOOD OF PCB CONGENERS IN RAINBOW TROUT *(Oncorhynchus mykiss)* SUSPENDED IN CAGES IN THE ST.LAWRENCE RIVER FRO 30 DAYS. Michael J. Kadlec and Brian Bush
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INTRODUCTION

Aquaculture in the St.Lawrence river could be a valuable source of cash income to populations living around the river such as the Mohawk nation at Akwesasne. It is possible to maintain large numbers of fish in large geodesic cages suspended in the water column of a large river, feed them automatically on a daily basis and harvest them at regular intervals. The problem with many rivers in the developed world is xenobiotic pollution: in the St.Lawrence river and Lake Ontario, PCB pollution is the major problem. PCB have until recently been considered to be sediment born, so that fish kept in isolation from bottom sediments might be isolated from the pollutant, also feeding clean chow should minimize predation on polluted biota and the fish may remain uncontaminated in spite of a generalized pollution of an aquatic environment. Uptake from the water column directly (bioconcentration) needs to be measured in isolation from food chain uptake (bioaccumulation). The experiment reported here is designed to do this using a commercial fish species *Oncorhynchus mykiss*, rainbow trout.

METHOD

Rainbow trout (30 cm) raised in a clean aquaculture environment were confined in caged suspended in the water column sites: Snye marsh (Adirondak river-fed control site) Reynold's Bay on the St.Lawrence river and Contaminant Cove which abuts a GM PCB-containing landfill. They were hand fed Purina Fish Chow daily in December 1992. Five fish were removed from each cage after 10, 20 and 30 days. Edible fillets were analyzed by homogenizing with sodium sulfate and hexane three times, purification on calibrated Florisil and analyzed by gas chromatography with electron capture detection using a Hewlett-Packard Ultra II 5% phenylmethyloctadecylsilyl quartz capillary column. 68 PCB-containing peaks, p,p'-DDE, mirex and HCB were calibrated for using a 1:1:1:1 mixture of Arochlors 1221, 1016, 1254 and 1260 (200 ng/mL of each) fortified with the pesticides at 10 ng/ mL (Bush et al., 1989). Data were sent electronically to a PC where they manipulated using Lotus 1-2-3 with a 3-D Graphics Version 1.01 (Intex Solutions Inc., Wellesley MA) add-on.

DISCUSSION

PCB do accumulate in fish from the water column alone. The concentration and PCB pattern accumulated at the most contaminated site greatly resembles the pattern in the water. There is little change in concentration between 20 and 30 days.

Bio-uptake factors follow the expect increase with degree of chlorination at the low water concentration sites but not in the heavily polluted site. It is possible that equilibrium had not been established at 30 days here. Bioaccumulation factors from food lie only between 0.5 and 2, whereas those from water lie between 3,000 and 30,000 making pollution of fish from the water column an important consideration in water pollution control.

Even fish from the highly contaminated site mostly fall below the USFDA recommendation against human consumption of 2 ppm (μg/ g).

CONCLUSION

The experiment shows that except at the very contaminated site where the water concentration exceeds 400 ng/ L of total PCB, raising trout for human consumption should be possible. The experiment was repeated during June 1993, to confirm this conclusion at summer water temperatures. The chemical analyses are not yet complete.

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ACKNOWLEDGEMENT

The authors wish to thank Tauni Houck for sample preparation and analysis and the USNIEHS for support from USNIEHS grand No. P42 ES04913-04.

MERCURY BIOACCUMULATION BY FISH AND CRABS IN A TEXAS BAY: A MODELING APPROACH*

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From 1966 to 1970 a chlor-alkali plant operated by ALCOA released mercury enriched wastewater into Lavaca Bay, Texas. A reservoir of mercury enriched sediment has persisted in parts of the bay. In 1988, the Texan Department of Health closed a portion of Lavaca Bay adjacent to the chlor-alkali plant to recreational and commercial taking of finfish and crabs, because the edible flesh of many of these organisms exceeded the FDA guideline for methylmercury of 1.0 ppm wet weight.

We searched the literature for information on the partitioning, bioconcentration, and bioaccumulation of inorganic mercury and methylmercury in aquatic ecosystems which could be used to define the connection between mercury contamination in the sediment and elevated mercury levels in Lavaca Bay biota. We have not considered other potential sources of continuing mercury contamination such as agricultural or urban runoff or industrial emissions. Additional information was sought on the life histories of fish and invertebrates found in bays and estuaries along the Texas coast that could explain this connection. Three target species, red drum, black drum and blue crab were chosen for development of a conceptual model of mercury bioaccumulation. Descriptive models of food web relationships were constructed which could be used to evaluate the possible pathways of mercury to these target species. In addition, models also were constructed for the major classes of primary food items: crustaceans, molluscs, and small fish. Models were conceptualized by boxes representing compartments of mercury accumulation and storage (e.g. sediments, water, fish, crabs, and molluscs) and arrows representing pathways of transfer of mercury among compartments.

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* - This study was funded in part by the Lavaca BAy Technical Management Team, NOAA/ Damage Assessment Center, and NOAA/ National Marine Fisheries Service.

In aquatic systems the form of mercury most readily bioaccumulated and which causes greatest toxic effect is the organic from, methylmercury. Methylmercury is emphasized in our model development for these reasons. We assume that sediments are the primary reservoir and potential source of mercury in Lavaca Bay. Past surveys have shown that a plume of mercury-enriched sediments emanated from the vicinity of the ALCOA chlor-alkali plant at Point Comfort, where more recent surveys have continued to show elevated concentrations of mercury in the sediments. The pathway of methylmercury from the sediments to target species is thought to be through feeding on sediment dwelling organisms such as crustaceans, molluscs, and worms. Another possible factor contributing to the transfer of mercury from the sediment to the biota is that the Lavaca Bay sediments are generally low in organic matter, which tends to bind methylmercury. While the chemistry of mercury in saline or estuarine waters is reasonably well understood qualitatively, actual measurements of mercury in estuarine waters are few, because the concentrations are very low and sample analysis is difficult. No data are available for dissolved methylmercury in Lavaca Bay. A water compartment is included in all of the descriptive models presented because of the potential role of water in transferring mercury from sediments to biota.

The two species of fish, red drum and black drum have life histories that help explain the observed differences in mercury concentrations in fish from estuaries and bays on the Texas coast, specifically the elevated mercury levels found in Lavaca Bay. Both species of fish tend to feed and grow in relatively restricted locations throughout the first three to five years of life. The result is that fish which reside in a location contaminated with mercury should have body burdens that reflect the environmental concentrations of mercury.

Red drum spend their first 3 to 5 years in coastal bays and estuaries where they grow rapidly. They stay relatively close to the area to which they originally recruited, reaching a total length of 600 to 700 mm. Based on our modelling, they receive their major input of methylmercury from food items rather than directly from either the sediment or the water. Their primary foods are crustaceans (crabs and shrimp). Small fish are also a food source of the red drum, but are probably not as important in the accumulation of mercury, since the methylmercury concentration in small prey fish is low relative to crustaceans.

Black drum have not been studied as extensively as red drum, but have a similar growth pattern, and they have a poorly understood age dependent migration pattern. They recruit into an estuary where they remain through their first five to six years. When the fish become sexually mature they may either spawn in the estuary in the vicinity of inlets or passes or move out into the Gulf of Mexico. Later, large adults may return to the same embayment. Lack of movement by the black drum during their early years and their return to the estuary reduced the uncertainty about where they acquire their food and associated mercury. The food chain supporting the black drum is simpler than the one that supports red drum. The two main sources of mercury are molluscs and crustaceans. Black drum is considered primarily a mollusc predator, since it is anatomically equipped to grind and crush mollusc shells.

The blue crab has a well defined life history, but a less specific habitat selection process than the two species of fish. It is more mobile than the fish. Blue crabs (along with stone crabs) are also among those species exceeding the FDA Guideline for methylmercury of 1.0 ppm wet weight in parts of Lavaca Bay. The molt cycle (i.e. the growth process) has been shown to affect metal metabolism in blue crab hemolymph and digestive gland, but it's effect on muscle methylmercury is not known. Blue crabs are opportunistic feeders with a diet that includes everything from dead red drum to juvenile oysters. They have been classified as detritivores, omnivores, and carnivores. Detritus, animal/plant material, sediment, and molluscs, are likely more important mercury sources than direct accumulation from water. Modelling mercury accumulation in blue crabs is the most complicated of the three target species, because feeding patterns are not well defined, growth is discontinuous and life span is short, and growth can directly affect the turnover and metabolism of accumulated mercury.

Major components of the food webs for red drum, black drum and blue crabs are actually assemblages of organisms (crustaceans, molluscs, and small fish). The descriptive models for these assemblages are more generic than the single species food webs. Crustaceans use animal and plant detritus, epiphytic and benthic algae, and benthic invertebrates (including both micro and macro-fauna) as food sources. The choice of particular organisms is dictated by their relative abundance and the developmental stage and size of the crab of shrimp. All species with the exception of xanthid crabs are found primarily in seagrass meadows and salt marshes where detritus is abundant. All are omnivores and detritivores. Molluscs have less complex food web relationships. Filter feeding molluscs can accumulate metals and nutrients both through the consumption of phytoplankton and directly from water. Sediment may be a dietary source of mercury for deposit feeding molluscs and an indirect one for filter feeders. Small fish are secondary to other more important food web pathways for the target species. Of all models, the small fish model has the highest level of uncertainty, because of the many species and their trophic diversities. there is a notable lack of information on their mercury concentrations in Lavaca Bay.

Implementation of the models and estimation of methylmercury bioaccumulation was dependent upon data taken from the world-wide mercury literature coupled to measured mercury and methylmercury concentrations in organisms and sediment from Lavaca Bay. Calculations were made of the fluxees of mercury through the food web to the three target species. Results of this implementation exercise were consistent with observed methylmercury concentrations in Lavaca Bay red drum, black drum, and blue crab. Results of the calculations illustrated the utility of the models, and identified the areas of uncertainty in model parameter estimates.

In summary, the modeling effort has identified five critical factors that have led to the localized elevations of mercury concentrations in certain finish and crustaceans in Lavaca Bay:

1. The persistence of high mercury concentrations in sediments in the Point Comfort area of Lavaca Bay.

2. Low organic matter concentrations in Lavaca Bay and Matagorda Bay sediments enhance mercury bioavailability to sediment inhabiting invertebrates.

3. The relatively simple food webs in which red drum, black drum, and blue crabs feed predominantly on benthic and epibenthic invertebrates.

4. Limited movement of red drum, black drum, and blue crabs within Lavaca Bay which allows therm to be exposed to the elevated mercury concentrations in these invertebrates

throughout most of their early lives.

5. Highly efficient and rapid assimilation of methylmercury from food by the target species. In combination with very slow excretion of methylmercury, this allows the target species to accumulate methylmercury to high levels during their residence in Lavaca Bay.

METALLOTHIONEINS AND TRACE METAL PARTITIONING IN THE ATLANTIC OYSTER, *Crassostrea virginica*

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The Atlantic oyster, *Crassostrea virginica*, has the capacity to concentrate trace metals from its environment to high body burdens as demonstrated in both field collections and in laboratory exposed animals. To cope with these accumulated metals, oysters possess efficient mechanisms to sequester and detoxify metals when present in excess or when required for routine metabolism. One mechanism to detoxify copper and zinc is the formation of intracellular inclusions in amoebocytes and excretion by diapedesis. Cytosolic cadmium sequestration in the oyster has been shown to involve the low molecular weight and metal-binding cytosolic protein, metallothionein (MT) (i.e., heat stable, molecular weight of 7 to 10 K daltons, binds cadmium, contains 30% cysteine, and has the amino acid sequence consistent with a Class I MT).

In our continuing investigation of metal metabolism in oysters, we have studied the uptake, accumulation, cytosolic partitioning, and depuration of cadmium, and how copper in the exposure media affects these processes with exposure to cadmium and zinc. The results of experiments where oysters were exposed simultaneously to cadmium and copper have shown that both metals were accumulated at similar rates. If only cadmium is removed from the exposure medium, the rate of copper accumulation doubles and cadmium is displaced from the MT by copper, but the total body burden of cadmium remains constent.

Gel filtration chromatography of the oyster cytosol showed the presence of two cadmium-binding peaks at 24K and 10K daltons molecular weight, containing cadmium, copper, and zinc. Ion-exchange chromatography of these two metal-binding protein peaks show that they are composed of at least two or three isoproteins. The ion-exchange elution profile of the higher molecular weight metal-binding peak shows the presence of a zinc containing protein and at least two ill-defined cadmium/copper proteins which do not have the characteristics of MT. The elution profile of the low molecular weight protein peak is composed of three isoproteins. Two of the proteins bind both cadmium and zinc while the third binds only zinc.

The elution characteristics of the two cadmium peaks are consistent with literature values for a Class I metallothionein.

Since environmental/monitoring measurements of MT in oysters use field collected specimens, we examined local nonexposed oysters at different times during the year and at different stages of their reproductive cycle. Examination of local winter oysters with background metal concentrations showed that the only metal bound to the MT was zinc. Ion-exchange separation of this material showed on predominant zinc containing peak and two shoulders. These three elution conductivities corresponded rather well with the two cadmium/zinc and one zinc protein discussed previously. These data suggest that the native form of MT in the oyster is Zn-MT, and that cadmium and copper can induce synthesis. In addition, the cytosol of prespawning and postspawning oysters was examined by gel filtration chromatography, and significant differences in the distribution of copper were demonstrated. These data underscore the need to be aware of the effects of natural factors on the metal concentrations in sentinel organisms.

In addition to influences of natural factors, population differences may also exist. In collaboration with colleagues at the University of Maryland and the NMFS/Milford Laboratory, we have examined the cytosolic distribution of metals in oysters from the northeastern and Southeaster U.S.. There are major differences among groups of oysters collected from estuaries along the east coast. Southeastern oysters have two metal-binding peaks at 24 and 10K daltons while the northeastern animals have one at about 10K daltons. Examination of these groups of oysters is still underway, but the early data suggests that there may population differences, which could in turn influence the accumulation of contaminants.

Like other biomarkers, MT will be useful for field monitoring when the effects of natural variables are understood. With the currently available molecular biological techniques, it should be incumbent upon any wide ranging monitoring program to characterize the population structure of the sentinel organism that is being used.

POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENT OF THE ST.LAWRENCE ESTUARY

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ABSTRACT

Elevated concentrations of polycyclic aromatic hydrocarbons (PAH) characteristic of combustion processes have been previously reported in the Saguenay Fjord and Baie des Anglais, and associated with the historical record of discharges by aluminium refineries. We analyzed PAH in sediments from the Saguenay Fjord (four cores) and the Lower St.Lawrence Estuary (15 locations). A dated Saguenay core contained total combustion PAH at concentrations of 1.9 to 160 μg/g which varied with time and sediment displacements. In the Lower Estuary, transects showed elevated levels of PAHs (up to 8.9 μg/g) near the north shore where a refinery is located. In the deep Laurentian Trough, however, PAH concentrations averaged around 1 μg/g (range 0.69 to 1.6 μg/g and showed no apparent geographic or temporal trends, indicating that the refineries have little influenced these sediments. The ratio of benzo(a)pyrene (a combustion PAH) to perylene (a PAH of similar physio-chemical properties with natural sources) was used to trace PAH from refineries by minimizing variability due to sedimentary grain size and organic carbon content. A series of PAH compounds derived from resin acids of terrestrial plants (norabieta-tetraene, norabieta-triene, tetrahydroretene and retene) was also prominent in the upper Saguenay core and may reflect the pulp and paper industry.

INTRODUCTION

Polycyclic aromatic hydrocarbons are of increasing concern because they are ubiquitously found in the environment (Laflamme and Hites, 1978; Gearing et al., 1991 and references therein) where they can have both short-term acute effects and long-term carcinogenic and mutagenic effects (National Research Council Canada, 1983; National Research Council, 1985). The larger PAHs are persistent in sediments. Their presence in marine environments has been linked to damage to fish, despite the ability of most vertebrates to metabolize these compounds (Malins et al., 1988).

Quebec has the highest yearly influx of PAHs in Canada, in large part due to the presence of several aluminium refineries (Lavalin Environment, 1988). High levels of PAHs in Quebec sediments have been reported, beginning in the early 1980s. This paper reviews that literature and adds more recently collected data to present a unified overview of PAHs in the St.Lawrence system.

MATERIALS AND METHODS

Three box cores were collected from the lower Saguenay Fjord in August, 1987. Samples from various depth intervals (0-0.2 cm, 0.2-0.5 cm, 0.5-1, 1-2, 2-5, 5-10, 10-15, 15-20, 20-25, etc.) were collected in solvent-cleaned glass jars and immediately frozen. A gravity core from near St.Fulgence was collected in February, 1990 and frozen whole. Sediments from the Lower St.Lawrence Estuary were collected with a box corer in May, 1985 (one, mid-estuary), in August, 1987 (two from the upper and lower sections of the Laurentian trough) an in July, 1989 (ten along two north-south transects). Box cores were subsampled as for the Saguenay cores and frozen on board ship.

In the laboratory, samples were thawed and sieved (82-μm mesh). Aliquots were taken for the determination of dry weights. Deuterated internal standards were mixed with the wet sediments. Lipids were extracted from the sediments by refluxing for 4 h in 1.5 M KOH/CH_3OH. The nonsaponified fraction which included the PAHs was separated by three liquid-liquid extractions with hexane. Sulfur was removed with activated copper. The hexane was concentrated and separated into aliphatic and aromatic fractions using columns of alumina and silica gel (Gearing et al. 1976).

PAHs were separated and tentatively identified by capillary gas chromatography using a Carlo-Erba HRGC 5160 with cool on-column injector, a DB-5 column (30 m x 0.32 mm ID), and FID detector. Quantification was carried out with a Spectra-Physics 4290 integrator coupled with the computerized Labnet/Winner system. Peak areas and heights were compared for quantification. Analyses by GC/MS (VG - TS250) on selected samples confirmed identifications and quantification.

RESULTS AND DISCUSSION

Saguenay Fjord

Three aluminum refineries operate along the Saguenay River and upper reaches of the Fjord. The surrounding highlands, prevailing winds and currents funnel the majority of atmospheric particulates down the Fjord where they can be deposited in the deep sedimentary basin.

The sediments of the fjord have been analyzed for PAHs since around 1979 (Table 1). These studies fall into two groups: a comprehensive set of cores and surface grabs taken between 1979 and 1983 at 47 sites and a smaller, more recent collection (1987-1991) from 19 stations. Taken together, these analysis show consistent geographical and temporal trends.

Table 1. Sediment samples from the Saguenay Fjord

Location	Sampling	Year	Reference
River and Tributaries	8 sites	1983	Paul & Laliberté, 1985
River	6 sites	1988	Laliberté, 1991
Baie des Ha!Ha!	12 grabs	1982	Primeau & Goulet, 1983
North Arm of Fjord	1 core	1979	Smith & Levy, 1990
North Arm of Fjord	1 core	1990	Pelletier et al, 1992
North Arm of Fjord	1 core	1990	This work
Upper Reaches & Main Basin	8 cores	1982	Martel et al., 1987
Upper Reaches & Main Basin	17 grabs	1983	Martel et al., 1986
Upper Reaches & Main Basin	3 cores	1988	Ouellet, 1990
Upper Reaches & Main Basin	5 cores	1991	DeMarco, 1992
Lower Fjord	3 cores	1987	This work

Results from these studies have been compiled on Figure 1. The term PAH combustion products has been used to refer to the sum of twelve unsubstituted PAHs [phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(c,d)pyrene, dibenz(a,c)anthracene and benzo(ghi)perylene]. This term comes from the geochemical literature. Various other combinations of unsubstituted PAHs are also reported in the literature and occasionally erroneously reported as "total" PAHs. The values reported in the above studies have been closely adjusted to this combination of compounds. Differences in methodology and chromatographic resolution precluded a complete coordination of values.

Values in the Saguenay River are generally low (1-2 μg/g) except in the immediate vicinity of refineries; there is little sedimentation in the river itself. In the upper reaches of the fjord near St.Fulgence, where sedimentation rates are up to 7 cm/yr and the sediments are anoxic (Smith and Walton, 1980), PAH concentrations are highest. Lower, but still elevated concentrations are measured in the oxic sediments elsewhere in the main basin, with the values declining with distance downstream. This decline becomes more pronounced 40 km downstream near the estuary, where the fjord becomes shallower, the bottom currents stronger, and the sediment grain size larger. Nevertheless, elevated PAH levels (> 2 μg/g) extend over 60 km down the fjord.

The concentrations of PAHs in these sediments show a small, but consistent decline with time. At three locations near the top, middle and lower sections of the main basin, values reported by Ouellet (1990), DeMarco (1992), Lambert and this work for 1987-1991 are 10 to 40% less than the values of Smith and Levy (1990) and Martel et al. (1987), for samples collected in 1979-1983. This corresponds to 1.1 to 8 percent loss per year with an average of 3% per year. Dated cores show that maximum values around 160 μg/g were found near St.Fulgence in the 1960s. Since aluminum refineries are currently working to reduce PAHs in their effluents, sedimentary levels can be expected to further decrease with time.

The combustion PAHs are not always the most abundant aromatic compounds in the fjord. A series of aromatic hydrocarbons derived from resin acids (primarily norabieta-tetraene, norabietatriene, tetrahydroretene and retene) have concentrations as high and sometimes higher than the primary combustion products. Preliminary analysis of their occurrence over time indicates that pulp and paper mills may be responsible for the high concentrations of these natural products.

St.Lawrence Estuary

There is also an aluminum refinery on Baie des Anglais, in the Lower Estuary. A recent study (MENVIQ, 1993) found elevated levels of combustion PAHs in the bay itself, up to 3 km from the refinery. We examined the literature and analyzed additional sediments in order to see how this source as well as inputs from the Saguenay affected the sedimentary levels of PAHs in the estuary as a whole. The samples surveyed are listed on Table 2.

In general, concentrations in the open estuary (Laurentian Trough) are relatively low, around 0.5 to 1 μg/g and do not change with distance down the estuary (Figure 2). In fact, levels reported for the St.Lawrence River (riverine lakes), the Upper Estuary and for the Gulf near the Cabot Strait fall in the same range. Higher values have only been reported for sediments in major harbours (Pelletier and Bélanger, 1990). In the Lower Estuary, only samples taken on the northern side of the estuary near Baie des Anglais, had values over 2 μg/g. The influence of the factory can be seen for tens of kilometres along the north shore where rivers ultimately deposit the PAH-laden particulates which were originally spread atmospherically. However, in contrast to the situation in the Saguenay, these particulates appear to have little influence on the deep depositional trough.

Table 2. Sediment samples from the St.Lawrence

Location	Sampling	Year	Reference
River - Lake St.François			Allan, 1988
River - Lake St.Pierre			Hardy et al, 1991
Upper Estuary - Basin	1 core	1986	Lambert et al.,unpub.
Upper Estuary - Interdidal	3 sites	1990	Dalcourt et al., 1991
LOWER ESTUARY			
Baie des Anglais	3 sites	1988	Laliberté, 1991
Baie des Anglais	17 grabs	1990	MENVIQ, 1993
Laurentian Trough	1 core	1985	Gearing et al., 1992
Laurentian Trough	4 grabs	1986	Gearing et al., 1993
Laurentian Trough	2 cores	1987	Lambert et al., unpub.
Laurentian Trough	1 core	1988	Ouellet, 1990
Interdidal - South Shore	7 samples	1986-89	Pelletier et al.,1991
North - South Transects	10 cores	1987	This work
Gulf - Basin	1 core	1988	Lambert et al., unpub.

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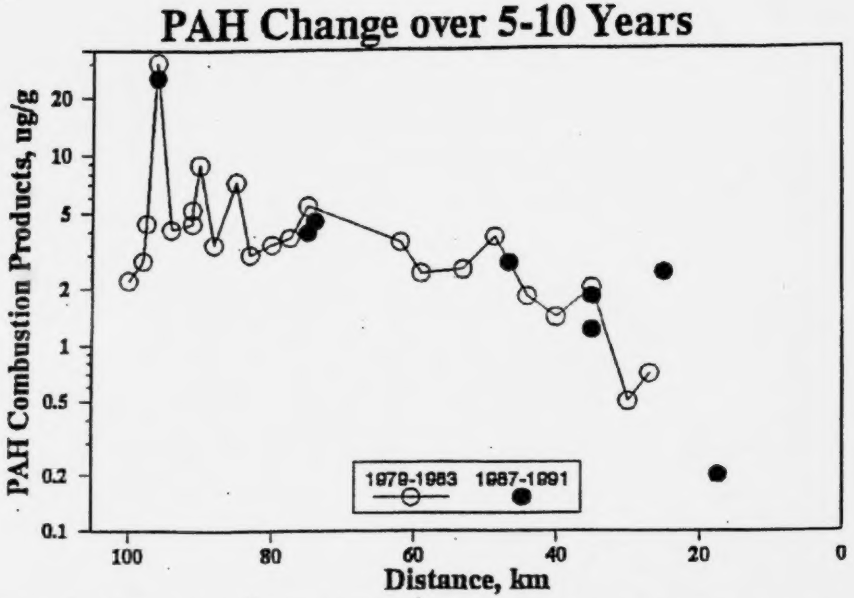

Figure 1. Total PAH combustion products (µg/g) in surface sediments of the Saguenay Fjord as a function of distance upstream from the estuary.

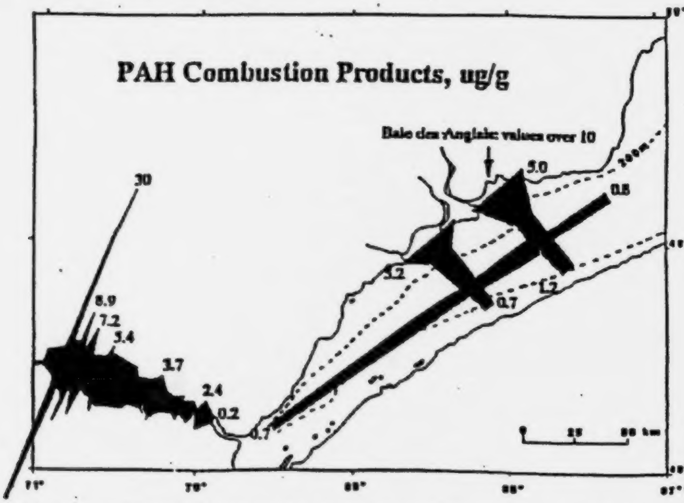
PAH Combustion Products, ug/g
Baie des Anglais: values over 10
30
8.9
7.2
3.4
3.7
2.4
0.2
0.7
3.2
5.0
0.8
0.7
1.2
200m
0
25
50 km
67°W

Figure 2. Total PAH combustion products (µg/g) in surface sediments throughout the Lower St. Lawrence Estuary.

CHALLENGES IN THE ASSESSMENT OF COMPLEX MIXTURES IN THE CANADIAN ENVIRONMENT

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INTRODUCTION

A few definitions are provided below to make the discussion more understandable.

A "CEPA substance" is defined as "any distinguishable kind of organic or inorganic matter, whether animate or inanimate", and includes:

- any matter that can be dispersed, or causes other matter to be dispersed or causes such transformations;
- free radicals and elements;
- any chemicals that do or dot not naturally occur;
- any class of substances.

This definition includes mixtures of substances, manufactured items that are used for their physical shape and any animate matter contained in effluents, emissions or wastes.

A "toxic" substance as defined by the Canadian Environmental Protection Act is: "a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions

a) having or that may have an immediate or long-term harful effect on the environment;
b) constituting or that may constitute a danger to the environment on which human life depends; or
c) constituting or that may constitute a danger in Canada to human life or health.

The use of the words toxic and toxicity in this paper refers to this definition only if they are followed by quotation marks. Other uses of these words refer to a more scientific definition.

A complex mixture could be defined as:

> a "CEPA" substance that is composed of many compounds, some that are closely related (for example PAHs), some that are not related (for example chlorinated phenols, heavy metals, and PAHs) and some that have not been identified or properly characterized (for example long chain aliphatics, soaps and azaarenes).

Four of the substances put on the Prioriy Substances List (PSL) under the Canadian Environmental Protection Act (CEPA) by the Hall Panel for "toxicity assessment" could be classified as complex mixtures, these were "Effluents from Pulp Mills Using Bleaching", "Chlorinated Wastewater Effluents", "Waste Crankcase Oils" and "Creoseoteimpregnatedf Waster Materials". These four substances result from specific industrial processes (pulp and paper mills and creosote wood preservation) or from urban development (waste crankcase oils and chlorinated wastewater effluents). Most of the other assessments conducted to date under the PSL are the products of many industrial processes (PAHs, benzene) or are individual chemicals (chromium, dichloromethane). There were some significant differences between the assessments of complex mixtures and those done on specific chemicals.

This presentation will identify the commonalities between the assessment of four complex mixtures and identify the challenges they presented to the assessment teams.

The basic problems in assessing a complex mixture are:

- characterizing the mixture

  Very large numbers of compounds can be present in a complex mixture. There are over 250 in bleached pulp mill effluents, hundreds in chlorinated wastewater effluents and waste crankcase oils, and up to 3000 in waste creosote. Complex mixtures can be highly variable over time and from one source to another. The complex nature of the mixture is such that the need to completely characterize it is not realistic.

- determining "toxicity"

  The toxic effects of complex mixtures can be difficult to determine as the many compounds in the mixture can exert toxic effects in different ways (sub-lethal mutations, direct damage, reduced reproduction, etc), they can interact with each other in unknown ways (antagonistic, synergistic, additive), and the effects can vary with time and source (increased toxicity in water, pulp effluent toxicity can vary with species of tree harvested, etc).

- determining the source of the "toxic" components

  Unlike single compounds, assessments of complex mixtures are source related, so determining the source of the components is very important in the assessment.

- fractionation of the mixture in the environment

  All of the mixtures fraction into at least several groups of compounds. Some are water soluble, some water insoluble; some degrade quickly, some persist; some are transported long distences, and others are immobile. Each mixture is unique in the way it fractions in the receiving environment.

CHARACTERIZING THE MIXTURE

Prior to conducting an environmental "toxicity" assessment of a complex mixture it is desirable do know what is in the mixture. Of the four examples used in this paper, creosote-impregnated waster materials was likely the easiest to characterize as it is formed from a commercial pesticide that has undergone considerable research characterizing its components for efficacy and for environment contamination. However, because creosote is made from coal it is highly variable in its composition. It includes PAHs, O, N, And S substituted PAHs, and alkylated phenols. Bleached pulp mill effluents typically contain resin acids, soaps, fatty acids, diterpene alcohols, phytosterols, chlorinated phenols and acids, alcohols, aldehydes, ketones, mercaptans, sugars and aliphatic and aromatic hydrocarbons. Waste crankcase oils contain many aliphatic and aromatic hydrocarbons, organic and inorganic compounds containing CI, S, P, Br, N and heavy metals (Pb, Zn, Ba, Mg) detergents, proprietary additives, dirt, and carbon. They are also frequently mixed with other waste oils that are not characterized at all. Chlorinated wastewater effluents can contain a wide range of compounds, but most common are PAHs, heavy metals, chlorinated phenols, free chlorine, nitrates, ammonia, phosphates, and salts. So as can be seen, the characterization of any of these "substances" is the environmental chemists nightmare come true, or perhaps their bread and butter.

Each complex mixture has of complicating factors that influence the composition of the mixture and hence the environmental toxicity. For example, the complicating factors in pulp mill effluents are the type of wood used (softwood produces more phenolic by-products that hardwood), in-plant processes (kraft vs sulphite, chlorine gas bleaching produces more chlorinated compounds than chlorine dioxide bleaching), and the effluent treatment process used. Waste crankcase oils are influenced by the manufacturer of the oil, additives in it, the way the vehicle was driven, the condition of the motor, the type of motor and the other waste oils mixed in. Chlorinated wastewater effluents are influenced by the type of effluent received (municipal vs industrial), the type of industry inputting to the system, the size of the population served, and the type of treatment system. The composition of waste creosote is mainly a product of the coal it derives from and the differences are usually in the percent distribution of the primary components.

Fortunately detailed characterization of a complex mixture is not necessary for a "toxicity" assessment as long as there is sufficient Canadian field effects data for that mixture. It is when the field effects data becomes limited, or of questionable quality, that effluent characterization becomes more important. In the assessment of creosote-impregnated waste materials, the characterization of the components aided the "toxicity" assessment. Knowing which PAHs from waste creosote partition onto sediments, water and aquatic biota aided the assessment by allowing a comparison of Canadian creosote contaminated sites to American sites where effects were noted and allowed the use of biological effects date (called AETs, Apparent Effects Thresholds) for single PAHs and total PAHs in sediments.

In the assessment of bleached pulp mill effluents, surrogate parameters were used to compare sources of effluents for similarity. This assessment used measurements of Adsorbable Organic Halogen (AOX) for the estimation of organochlorine content in mill effluents and receiving waters, and Extractable Organic Chlorine (EOCl) for sediments and biota. Much of the foreign literature on the effects of pulp effluents used these surrogate measurements. AOX was a useful parameter to determine the total amount of chlorinated substances in pulp mill effluents, but it could not be used to: distinguish one chlorinated compounds from another; provide the concentration of each chemical in the effluent; predict the toxicity of the effluent and predict the persistence of the effluent in the receiving environment.

DETERMINING "TOXICITY"

It is impossible to assess the "toxicity" posed by all of the individual components in a complex mixture as there are too many interacting parameters to consider. There are the problems of additive, synergistic and antagonistic effects of target components in mixture, and determination of the target species affected. The ecosystems being impacted are complex and much more difficult to study than a laboratory experiment or microcosm. The species exposed to the mixture react in unique ways depending on their physiology, reproductive status, sex, age and other factors. However, the best data to base a "toxicity" assessment on are negative biological effects data from Canadian sources of the complex mixture. If these are available then the composition of the mixture is of secondary importance to a "toxicity" assessment. If Canadian field effects data are not available then field effects data from similar systems in similar countries can be used to compare with the Canadian situation, assuming that the sources can be compared to establish similarity. Failing the availability of foreign effects data, laboratory toxicity data of the mixture can be used, but with considerable caution. Laboratory data are best used in support of sparse or questionable field effects data. Failing the availability of laboratory data on the complete mixture, then laboratory data on the component chemicals of a mixture can be used with caution and could not be used solely to support a decision of CEPA "toxicity". Field effects data from poorly related ecosystems can be used to support limited or questionable Canadian field effects data, but they too are not an indication of "toxicity" in the Canadian environment.

Finding scientific literature for field effects data from Canadian sources was readily accomplished for chlorinated wastewater effluents, as there was considerable information on impacts under Canadian conditions. Unfortunately, there was less information available on the impacts of creosote-contaminated waste materials on Canadian ecosystems, there was hardly any available information on bleached pulp mill effluents, and there was no information on waste crankcase oils. The evaluators had to use other sources of information for these three assessments. For the information base of bleached pulp mill effluents, the Swedish mills and receiving environments were similar to Canadian mills and situations, and for creosote-contaminated waste materials some of the American contaminated sites were sufficiently similar to Canadian ones to make comparisons. For waste crankcase oils there were no sources of information on environmental impacts.

A technique that worked well on the assessment of creosote-contaminated waste materials was to concentrate the assessment on a suite of 16 PAHs known to occur in all sources of creosote-contaminated waste materials, and that various environmental agencies had identified as "toxic substances" of are "priority pollutants". The PAHs considered covered the major components of waste creosote, so that most of the environmental behaviours of waste creosote, (i.e. fractioning into two distinct phases in water) were considered. However, this meant that toxicity information on compounds occasionally found in creosote or incomplete toxicity data on other commonly present compounds was not considered. Limited data indicated that much of the toxicity of waste creosote could come from the more water soluble quinolines. Unfortunately, most of the sampling programs at creosote contaminated sites did not include the quinolines as important components of waste creosote, so there was little data that could be used to compare effects seen at one site with the component chemistry to other sites.

In the assessment of bleached pulp mill effluents the concentrations of suspected toxic components were typically below their 96 hour LC50s, however, the toxicity of the effluents were typically high. If only the LC50 values had been used to determine the "toxicity" of bleached pulp mill effluents then the assessment would likely have been much different. This phenomenon is the major fault of using laboratory derived data on isolated components of a complex mixture. The information frequently does not adequately reflect reality.

SOURCE OF TOXIC COMPONENTS

The basic difference between assessing a simple compound and a complex mixture is in determining the source of the substance. The presence and toxic effects of a single compound or a simple mixture of related compounds can be assessed. The comlex mixtures assessed to date have been source specific, so that the presence of toxic components in the environment had to be continually questioned as to their source. For example, the presence of PAHs in a river was not adequate for the creosote-impregnated waste materials assessment, the PAHs had to be tied to a source of waste creosote. With some of the complex mixtures (creosote-contaminated waste materials and waste crankcase oils) there were problems with determining the source of toxic components in the receiving environment. In the case of waste crankcase oils, it was impossible to show the entry of waste crankcase oils into the aquatic environment. Heavy metals and PAHs are common pollutants in waters receiving industrial and municipal effluents and there is no specific component of waste crankcase oils that would have identified it in the environment. There are many sources of PAHs in aquatic environmentals other than creosote contaminated sites, such as sewage treatment plants, runoff from roads, oil spills, etc. This is problematic with assessments of pollutants from non-point sources as it may not be evident that the complex mixture under assessment is responsible for the negative environmental effects. Biological effects data have to be gathered from point sources where the concentrations of potentially toxic compounds from the complex mixture overshadow the background concentrations.

FRACTIONING IN THE ENVIRONMENT

All of the complex mixtures dealt with under PSL 1 fractioned in some way once they were released to the environment and created another confounding factor in determining if a mixture was "toxic" in the environment. Unlike a single compound where a limited series of degradation products have to be considered, complex mixtures degrade or fraction in a myriad of ways. These CEPA "substances" have many different routes of environmental toxicity with different, and variable endpoints. With waste creosote the 2-ring PAHs, phenols and azaarenes are the first to evaporate, dissolve or leach leaving behind the heavier multi-ring compounds. This creates two fractions each with a different toxicity potential and with different patterns of mobility in the environment. Chlorinated wastewater effluents and bleached pulp mull effluents are partially degraded before they are released to the environment. Bleached pulp mill effluents are detected in organisms 50 or more kilometres from their source while others degrade rapidly. Readily degraded compounds can exert an anoxic effect on the bottom organisms immediately downstream from the effluent outfall, while persistent bioaccumulative components exert their effects much further downstream on different organisms and over a longer time period. To be complete a "toxicity" assessment should take into account as many of these routes as possible, although time constraints and availability of information usually limit assessments to the most obvious routes of toxicity.

CONCLUSIONS

Complex mixtures are a common occurrence in the Canadian environment. Almost every industrial effluent and many wastes are complex mixtures. Determining their environmental impacts is more difficult than for single compounds due primarily to the high variability in their composition. This makes it difficult to detect or predict their toxicity, persistence and fate in receiving ecosystems. Careful thought should be given to conducting more assessments on complex mixtures without some pre-assessment work to determine if the proposed assessment is necessary. In many cases, the mixtures contain individual compounds that are more readily assessed and would result in much the same level of environmental protection.

The most important conclusion is that Canadian field effects data is much simpler to use for the simple fact that it does not have to be compared in any way with any data from another site or country, surrogate parameters do not have to be invented to compare similarities of effluents, lab data does not have to be extrapolated to the field, and there are no concerns about testing non-native species. There are many drawbacks to using field date, the inherent variability and unpredictability of natural ecosystems are the biggest drawbacks. But a well designed impact study is not difficult to plan and conduct, and the usefulness of the data is much greater than a lab study. The great value that evaluators put on field effects data of pollutants on Canadian ecosystems in assessing impacts is proof that if we want to adequately assess our impacts on the Canadian environment then we must have Canadian effects data. If we turn away from conducting field studies of impacted Canadian ecosystems, then we will not have the information we need to assess or control pollutants. Using foreign sources of ecosystem impact data is at best educated guesswork when applied to Canadian ecosystems.

BIODEGRADATION OF PENTACHLOROPHENOL AND BTEX IN A LAB-SCALE FLUIDIZED BED BIOREACTOR Jiyu Lei and Jean-Luc Sansregret, BIOGENIE INC. 350, rue Franquet, entrée 10, Sainte-Foy, (Québec), G1P 4P3

ABSTRACT

The biodegradation of pentachlorophenol (PCP) and some monocyclic aromatic hydrocarbons (BTEX) was investigated in a three-phase fluidized bed bioreactor. Support particles (polymers) of the reactor, possess a large surface area on which either a PCP of a BTEX degrading bacterial consortium was immobilized. The consortia, obtained by enrichment from chemical contaminated soils, are capable of growing in a medium containing inorganic salts and target contaminants as sole source of carbon. Biodegradation tests were performed using wood washing process water contaminated with PCP and groundwater contaminated with BTEX.

Results of the tests showed (greater than 99%) removal of PCP and BTEX to their analytical detection limits with a hydraulic retention time of less than two hours. A mass balance was made to measure the concentration of contaminants in inlet and outlet waters, gas emission and bed media. The balance study showed that biodegradation is a major reaction responsible for the contaminants removal Based upon this investigation, we conclude that the fluidized bed bioreactor would be effective for the treatment of organic compounds in wastewater.

KEYWORDS

Fluidized bed bioreactor, immobilization, biodegradation, pentachlorophenol, monocyclic aromatic hydrocarbons.

INTRODUCTION

Fluidized bed bioreactor (FBB) has potentially many applications in wastewater treatment owing to its advantages over other types of biotreatment (activated sludge, trickling filter, rotating contactor), such as: 1) high cell concentration; 2) low wash out of biomass; 3) high mass transfer and 4) minimum clogging of packing particles.

In FBB, packing particles are kept in a mobile state by the flow of gas and liquid. Microorganisms can be immobilized in or on the fluidized particles at a high concentration by covalent coupling, adsorption or entrapment. Wastewater treatment in such a reactor involves the bioconversion of pollutants to some non-toxic products by the immobilized microorganisms.

FBB has been used extensively for biological nitrification, denitrification and BOD removal. Preview research showed an important increase in the pollutants removal efficiency in FBB compared to some conventional biological treatment processes (Gauntlett, 1981; Sutton & Mishra, 1990; Cooper & Williams, 1990). So far, however, FBB has been applied to a relatively lower extent for the biotreatment of industrial chemicals contaminated waters. Due to its unique characteristics and various advantages, FBB is likely to find more widespread applications in the near future.

In the present work, a laboratory-scale FBB has been tested for the biodegradation of PCP (pentachlorophenol) and BTEX (benzene, toluene, ethylbenzene and xylenes), chemicals frequently found in industrial process wastewaters.

MATERIAL AND METHODS

Wastewaters

Washing process liquid of PCP contaminated wood was used as PCP contaminated water. The initial concentration of PCP was 40,5 mg/liter.

BTEX contaminated water was collected from the top of the water table at a gasoline contaminated site. An initial BTEX concentration of 731 μg/liter was measured, including 270 μg/liter of benzene, 260 μg/liter of toluene, 36 μg/liter of ethylbenzene and 165 μg/liter of xylenes.

Fluidized bed bioreactor

Fluidized bed bioreactor used for this study consisted of a 2-liter column, as shown in figure 1. The column was filled with biomass support particles possessing a high volumetric surface and having a density close to water. Compressed air was introduced from the base of the reactor through a porous diffuser. Wastewaters were injected into the reactor by a peristaltic pump and flowed concurrently with air upward. The liquid and air flows were the driving forces inducing the fluidization of the packing particles. Before being introduced into the reactor, pH and nutrients of the wastewaters were adjusted in a feed tank.

Microorganisms

PCP degrading bacterial consortium was isolated from the chemical contaminated soils and subcultured for several months in a mineral salts medium containing PCP as the sole carbon source. Shake flask experiments were conducted to evaluate PCP degrading activity of the consortium. Flasks containing 200 ml of mineral salts medium with 100 mg of NaPCP per liter were inoculated with 10 ml of the bacterial culture. Non-inoculated medium was used as control to check for the possible chemical and physical loss of PCP. All the flasks were incubated at 25°C on a shaker. PCP concentrations were analyzed according to Fountaine et al. (1975) using an UV/VIS spectrophotometer at 320 nm.

Bacteria for the degradation of BTEX were obtained from gasoline soils. The bacterial mixture was maintained in a mineral salts medium with gasoline as the sole carbone source. The BTEX degrading capacity of the consortium was determined by colony hybridization using the *xyl*E gene probe (Samson et al., 1992).

Experiments with the fluidized bed bioreactor

Experiments were conducted to investigate the biodegradation of PCP and BTEX in the FBB. PCP or BTEX utilizing consortium was immobilized in the support particles of the reactors. For BTEX degradation experiments, an activated carbone column was connected to the outlet of the air line for the adsorption of BTEX1. PCP and BTEX were measured by gas chromatography using standard EPA methods No 8040 and No 8020, respectively (U.S. EPA, 1986).

RESULTS AND DISCUSSION

Biodegradation of Pentachlorophenol

After several months of growth on pentachlorophenol, the PCP degrading consortium consisted of three predominant bacterial strains. Shake flask experiments showed that about 90% of NaPCP was degraded by the consortium in five days while in the control flasks (non-inoculated), no apparent loss of PCP was observed (Fig. 2).

The start-up of the luidized bed bioreactor consisted of a batch culture of the PCP degrading bacteria in the reactor for one day, time during which the bacteria could adapt to the wastewater and adhere to the surface of biomass support particles. Following the start-up phase, the reactor was then operated under a semi-continuous mode for thirty-four days. A control reactor without inoculation was run in parallel. Figure 3 shows the % of PCP removal efficiency based on influent and effluent PCP concentrations.

In the control reactor, the PCP removal was superior to 95% during the first two days and was then dramatically reduced. It is believed that the support particles had some adsorption capacity of PCP and was saturated with PCP after two days. In contrast, in the reactor inoculated with the PCP degrading consortium, the percentage of PCP removal remained relatively constant (from 99.0% to 99.9%) throughout the experiment. Data obtained suggest that the bacterial acclimatation period is short (<4 days) and that biodegradation plays a major role in the PCP removal from the water, since several days after the start-up of the system, the physical and chemical removal of PCP is no longer significant.

Biodegradation of BTEX

The gasoline-utilizing consortium, after subcultures in a medium with gasoline as the sole carbon source, was composed of five predominant bacterial strains. BTEX degrading capacity of the consortium was evaluated by colony hybridization using the *xylE* gene probe. The *xylE* gene, derived from ***Pseudomonas putida*** ATCC 32015, code for catechol-2,3-dioxygenase which is involved in the biodegradation of BTEX. The gene probe test showed that two strains in the bacterial culture were *xylE* positive.

Similar to the start-up for PCP degradation experiments, the BTEX degradation consortium was inoculated into the reactor and developed for one day in the support particles. The BTEX contaminated water was then pumped from the base of the reactor continuously through a peristaltic pump. The experiments lasted 14 days. Figure 4 shows the performance of the reactor for BTEX removal. Results demonstrated that once the system was in steady state, the BTEX removal efficiency was greather than 99%.

At the end of experiments, BTEX concentrations were analyzed in the packing material of the reactor and in the activated carbon. A mass balance study was made to estimate the BTEX fate during the experiments. The study showed a mass distribution as follows: 97.5% loss of BTEX by biodegradation, 1.9% by volatilization and 0.6% remained in outlet effluent (Fig.5).

One of conventional wastewater treatment technologies is adsorption on granular activated carbon. Using this method, high removal efficiencies are achievable for some organic pollutants such as BTEX. However, it has several disadvantages, including: 1) pollutants are only transferred from water to carbon; 2) only a limited number of pollutants can be efficiently eliminated by activated carbon adsorption and, 3) treatment cost may be high, particularly in the case of high concentrations of pollutants. Recently, an increased use of biological treatment has been observed. Fixed-film bioreactors (trickling filter) submerged packing reactor) have been developed and used at pilot-scales for the treatment of effluent containing BTEX and PCP. Removal efficiencies greater than 95% have been achieved with these systems (U.S. EPA, 1991; Biotreatment News, 1993; Larsen & M. Nielson, 1993). The percentage of BTEX and PCP elimination observed during our study in the FBB were found to be comparable to those obtained by the above-mentioned systems.

CONCLUSIONS

The preliminary results of PCP and BTEX biodegradation obtained in a laboratory-scale FBB are encouraging. However, before this technology is applied efficiently and cost effectively at a large scale, more detailed studies should be done. These studies could include the determination of basic design and operational parameters, and the technical and economic assessments of the FBB system in comparison with currently used methods (i.e. activated carbon adsorption and biological fixed bed reactors).

ACKNOWLEDGMENTS

The gene probe test was conducted in Dr. Denis RHO's laboratory at Biotechnology Research Institute of CANADA, Montreal. We gratefully acknowledge his contribution. We would like to extend our appreciations to our skillful technicians for their enormous laboratory efforts.

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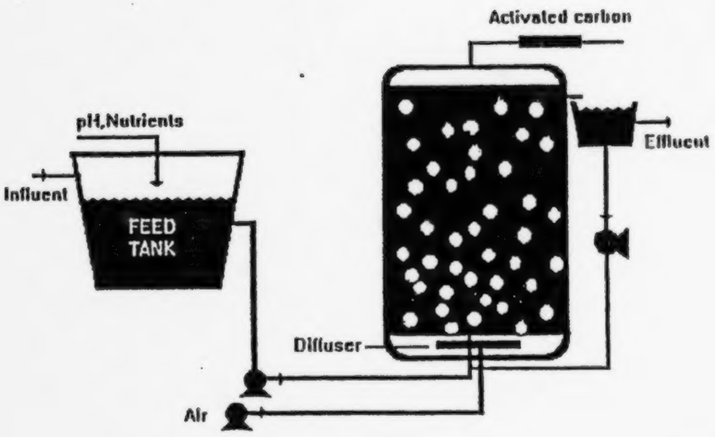
Activated carbon
pH,Nutrients
Influent
FEED
TANK
Effluent
Diffuser
Air

Fig.1: Diagram of the experimental apparatus

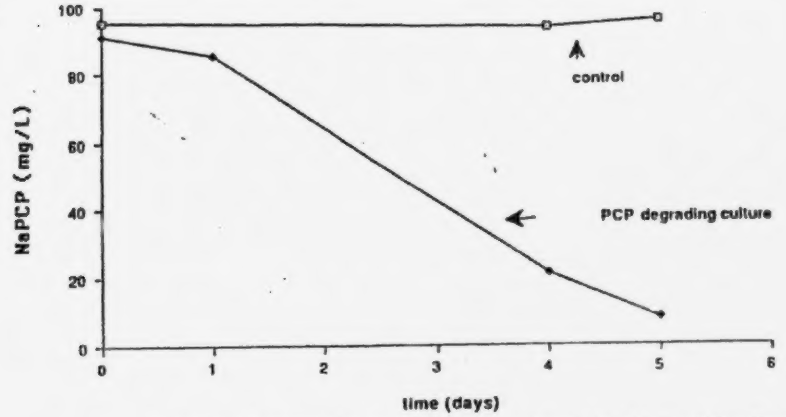

Fig.2: PCP degradation by the PCP degrading bacterial culture.

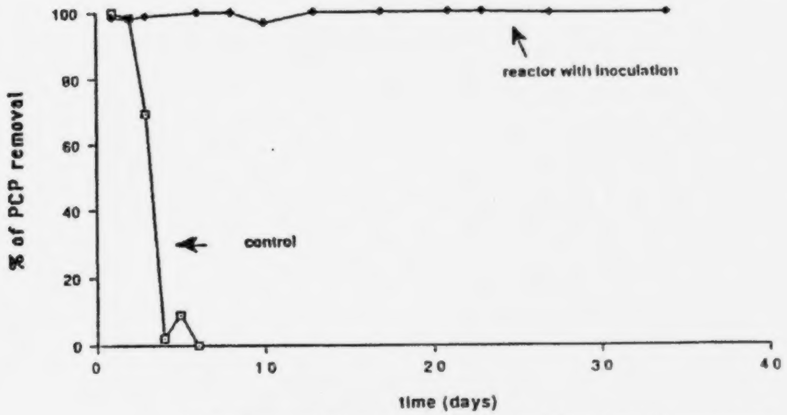

Fig 3: Percent removal of PCP in fluidized bed bioreactors.

aluminum and cadmium, are non-essential elements.

The results of 5-day growth bioassays confirm that both species are acid-tolerant, growing optimally at pHs < 4. In fact, at pH 2.5 to 3, in the absence of elevated metal concentrations, *E. gracilis* outcompetes *E. mutabilis* and achieves much higher cell densities. Therefore, low pH is not limiting to the growth of either species, a finding which supports literature reports that euglenoids are generally very tolerant on acidic conditions.

E. mutabilis demonstrates greater resistance than *E. gracilis* to zinc and aluminum in long-term (5-day) growth bioassays. High concentrations of zinc (up to 10 mM) and aluminum (up to 50 mM) have little impact on the growth of *E. mutabilis*. Growth of *E. gracilis* is significantly inhibited at much lower concentrations of these metals (e.g. 1 mM Zn and 10 mM Al).

Both species react similarly to copper and cadmium. Copper concentrations above 1 mM are inhibitory to growth causing > 50% growth depression (compared to controls). In fact, *E. mutabilis* appears to be more sensitive to copper than *E. gracilis*. Cadmium is the most toxic element tested causing deleterious effects at concentrations as low as 0.01 mM.

For the four metals tested, the order of increasing toxicity is: Al < Zn < Cu < Cd; this pattern is similar for both *Euglena* species. Therefore, the differences in growth responses of the two euglenoids are related to the concentrations of the metals present rather than the type of metal. Clearly, the elevated metal concentrations characteristic of AMD are influential in determining the species of organisms present. The results of this study demonstrate that differential metal resistance between these two euglenoid species, alone, accounts for their ecological distributions in nature. The underlying physiological/biochemical mechanisms permitting the observed sensitivity to some metals (e.g. copper, cadmium) and resistance to others (e.g. aluminum, zinc) remain to be elucidated. The euglenoids used in this study offer a unique "model" for examining metal tolerance mechanisms with minimal interference of pH fluctuations and concomitant changes in metal speciation.

ANALYSE DES FACTEURS BIOTIQUES ET ABIOTIQUES INFLUENÇANT LA BIOACCUMULATION DES MÉTAUX TRACES, CHEZ BITHYNIA TENTACULATA (MOLLUSCA: GASTROPODA) DU LAC ST-LOUIS (QUÉBEC) Christiane Flessas(1), Bernadette Pinel-Alloul(1) et Peter G.C. Campbell(2) (1) Département des sciences biologiques, GRIL, Université de Montréal, C.P. 6128, Montréal (Québec), H3C 3J7 (2) Université du Québec, Institut National de la Recherche Scientifique, INRS-eau, C.P. 7500, Sainte-Foy.

RÉSUMÉ

Plusieurs facteurs influencent l'incorporation et la bioaccumulation des métaux par les organismes aquatiques, citons par exemple la répartition des métaux dans les différentes phases géochimiques des sédiments, la biodisponibilité des métaux, ainsi que de nombreux facteurs environnementaux (matière organique, acidité, dureté, etc...). Dans le but d'évaluer le potentiel du gastéropode, *Bithynia tentaculata* pour le développement d'indicateurs de la qualité du milieu, nous avons mis en relation les concentrations de métaux dans les gastéropodes, avec celles retrouvées dans les divers compartiments biologiques (seston, périphyton, macrophytes) ainsi que plusieurs variables physico-chimiques de l'eau et des sédiments. Nous avons élaboré des modèles de prédiction des concentrations de Cd, Cr, Fe, Mn et Zn dans les gastéropodes à l'aide de plusieurs variables (modèles de régression multiples); ces modèles expliquent de 55% à 94% de la variance observée. Pour certains métaux, la taille des organismes (juvéniles ou adultes) ainsi que la période d'échantillonnage (juillet et août) ont apporté plus d'explication à nos modèles. Aucune relation n'a pu être établie dans les cas du Cu, Ni et Pb.

ABSTRACT

Numerous factors are known to influence the uptake and accumulation of metals by aquatic organisms, like the bioavailability of metals, the concentrations of metals in the different geochemical fractions of the sediments, as well as some other environmental factors (organic matter in sediments, acidity, water hardness,...). In attempt to evaluate the potential of a specie of gastropod (*Bithynia tentaculata*) for the development of an indicator of water quality, we put in relation the metal concentrations in gastropods with the ones observed in seston, periphyton and macrophytes, as well as several physico-chemical factors of water and sediments. We found significant relationships and eleborated predictive models for Cd, Cr, Fe, Mn and Zn, using a combination of variables (multiple regressions). These models explained 55% to 94% of the observed variations. For certain metals, the size of the organisms (juvenils and adults), as well as the sampling period (July and August), added additional explained variance in the models. No significant relationship was found for Cu, Ni and Pb.

INTRODUCTION

Aujourd'hui, il est bien connu que plusieurs espèces d'invertébrés, dont les mollusques, sont capables d'accumuler de grandes concentrations en métaux (Berger et Dallinger 1993). Les mollusques bivalves, en particulier, ont très souvent été utilisés comme bioindicateurs quantitatifs de la pollution métallique. Les moules marines comme *Mytilys spp*. ou d'eau douce comme *Lampsilis ventricosa, Unio pictorum, Elliptio complanata* et *Corbicula spp*. ont été utilisées avec succès dans des programmes de surveillance environnementale (Programme Mussel Watch par exemple) (Berger et Dallinger 1993; Berkman et Nigro 1992; Tessier et al., 1984). Les gastéropodes ont par contre été peu étudiés, que ce soit les espèces terrestres, dulcicoles ou marines (Berger et Dallinger, 1993).

Le but de notre étude était donc d'évaluer la possibilité de pouvoir utiliser un mollusque gastéropode, *Bithynia tentaculata*, en tant que bioindicateur de la contamination métallique dans un écosystème d'eau douce: le lac Saint-Louis, un lac fluvial du Saint-Laurent.

A *priori* nous avons considéré les gastéropodes pour leur potentiel d'utilisation à titre de bioindicateur parce qu'ils sont très répandus le long du couloir fluvial, qu'ils sont faciles à échantillonner et qu'ils jouent un rôle important dans l'alimentation des poissons (Caims et Pratt 1993).

Selon Davies (1992) le défi des années 1990 dans la recherche sur les métaux traces est de dépasser les simples relevés de niveaux de contaminants, et de développer des modèles de prédiction de la bioaccumulation et du transfert de ces contaminants. En effet, le développement de solutions efficaces et économiques pour résoudre les problèmes de contamination des cours d'eau dépendra surtout de notre capacité à prédire comment les mécanismes de dépollution vont améliorer la qualité du milieu et à définir les effets des changements écotoxicologiques sur les organismes aquatiques (Tessier et al., 1993). Notre projet répond à ces objectifs et s'insère dans le cadre du Plan d'Action Saint-Laurent, qui vise l'élimination de 90% des rejets toxiques des cinquante industries les plus polluantes du fleuve. L'utilisation d'outils de surveillance environnementale s'avère donc indispensable, pour établir un suivi de l'état de santé du fleuve. Les mollusques unionides ont été souvent proposés comme organisme-sentinelle de la pollution métallique (Caims 1990; Caims et Pratt, 1993), mais c'est la première fois que les gastéropodes sont utilisés en tant que bioindicateurs de la contamination métallique dans le fleuve Saint-Laurent.

Ainsi, si nous arrivons à expliquer, du moins en partie, la contamination métallique des gastéropodes par diverses variables biotiques et abiotiques du milieu, *Bithynia tentaculata* pourrait servir d'outil de surveillance des niveaux de contamination métallique de l'écosystème fluvial. Pour arriver à prédire de façon réaliste l'impact des métaux sur les organismes aquatiques, il est nécessaire de comprendre comment les facteurs physiques et chimiques agissent sur l'assimilation des métaux par les organismes (Bourgouin et al, 1991; Luoma 1983). Les effets de ces facteurs sont encore mal compris et des relations simples entre les concentrations métalliques des organismes et les concentrations totales en métaux dans les sédiments ou dans l'eau, sont rarement retrouvées dans les écosystèmes naturels (Bourgouin et al, 1991).

Nous posons quatre hypothèses pour arriver à élaborer des modèles de prédiction de la contamination métallique chez le gastéropode *Bithynia tentaculata*. Tout d'abord, (1) les facteurs abiotiques tels que la contamination des sédiments, de l'eau interstitielle (modèle de l'ion libre) ainsi que d'autres facteurs physico-chimiques de l'eau et des sédiments peuvent contribuer au transfert et à la bioaccumulation des métaux par les gastéropodes. Toutefois, les facteurs abiotiques n'expliquent pas toute la variabilité observée dans la contamination des organismes et (2) certains facteurs biotiques (concentrations en métaux dans le seston, le périphyton et les macrophytes) peuvent aussi agir sur la contamination des gastéropodes et même avoir un effet plus important ou additionnel à ceux des facteurs abiotiques. Finalement, deux autres facteurs liés à la croissance des organismes et aux changements temporels à court terme dans les conditions environnementales peuvent aussi moduler la bioaccumulation des métaux. Ainsi, nous postulons que (3) la taille des gastéropodes influence leurs niveaux de contamination métallique et que (4) les niveaux de contamination métallique des gastéropodes varient au cours de l'été.

MATÉRIEL ET MÉTHODES

Bioindicateur:

L'organisme cible que nous avons utilisé est un gastéropode prosobranche, *Bithynia tentaculata* (figure 1), dont la taille peut atteindre 13 mm (Clarke 1981). Cette espèce introduite, originaire d'Europe est aujourd'hui largement répandue dans le bassin inférieur des Grands Lacs et du Fleuve Saint-Laurent, où on la retrouve en eau peu profonde. Elle est qualifiée d'espèce ubiquiste (Clarke 1981). Son cycle de développement annuel a été décrit par Pinel-Alloul et Magnin (1971).

Site d'étude:

Le lac Saint-Louis est un élargissement du fleuve Saint-Laurent situé en amont de Montréal, au confluent de deux masses d'eau: les eaux brunes originaires de la rivière des Outaouais au nord et les eaux vertes en provenance des Grands Lacs au sud. Ces deux masses d'eau possèdent différentes propriétés physico-chimiques. Ainsi, les eaux brunes situées au nord sont peu minéralisées et plus acides que les eaux vertes du sud (Sylvestre et al, 1992).

Échantillonnage:

L'échantillonnage a eu lieu à deux reprises au cours de l'été 1991, soit du 15 au 25 juillet et du 12 au 22 août. Douze stations ont été choisies en fonction d'un gradient de contamination dans les sédiments (figure 2). Certaines stations se trouvent dans les eaux brunes sous l'influence de la rivière des Outaouais (1, 3, 4 et 5) ou dans la zone d'influence de la rivière Saint-Louis (7, 8, 11 et 12) et les autres dans les eaux vertes du Fleuve Saint-Laurent (2 et 6).

Plusieurs données physico-chimiques de l'eau ont été mesurées sur le terrain (soit le pH, Ca^{2+}, la température, l'oxygène, ...). Les sédiments ont été échantillonnés par des plongeurs à l'aide de carottiers en polypropylène. Les gastéropodes ont été récoltés à l'aide de filets troubleau, triés par espèce et par classe de taille, puis congelés jusqu'à l'analyse. Les macrophytes (partie verte seulement) ont été recueillis par des plongeurs et placés avec de l'eau du lac dans des sacs en plastique. L'échantillonnage du périphyton a été fait à l'aide de "boîtes à périphyton". Ces boîtes en Plexigas™, manipulées par des plongeurs, ont été refermées sur les macrophytes. Une fois à la surface, le contenu des boîtes a été vidé dans un seau puis brassé vigoureusement. Ceci a pour effet de déloger les algues fixées à la surface des plantes, qui ont été ensuite retirées pour ne conserver que la solution de périphyton. Le seston (ou matière en suspension dans l'eau) a été récolté par filtration d'une grande quantité d'eau (environ 25 litres) pompée mécaniquement.

Analyses de laboratoire:

L'analyse des métaux dans les sédiments a été réalisée à l'aide d'une procédure d'extraction séquentielle, développée par Tessier et al, 1979., qui consiste à utiliser des réactifs de plus en plus puissants afin d'extraire successivement les métaux liés aux différentes phases géochimiques. Les solutions de ces extractions ont par la suite été dosées pour leur contenu en métaux, par spectrométrie d'émission atomique au plasma (I.C.P) et par spectrophotométrie d'absorption atomique à la flamme et au four au graphite. Les concentrations en métaux obtenues correspondent aux concentrations de métaux liés à quatre phases géochimiques des sédiments.

MF1: Métaux échangeables;
MF2: Métaux liés aux carbonates ou spécifiquement adsorbés;
MF3: Métaux liés aux oxydes de fer et de manganèse;
MF4: Métaux liés à la matière organique et aux sulfures.

Les gastéropodes ont été retirés de leur coquille et les tissus mous des gastéropodes, ont été séchés, digérés dans l'acide nitrique, puis analysés pour leur contenu en métaux par spectrophotométrie d'absorption atomique (four au graphite et à la flamme). Les macrophytes ont été triées par espèce, lavés à l'eau déminéralisés, séchés, puis broyés. Ils ont subi une digestion à l'acide nitrique. Le dosage des métaux a également été effectué au four au graphite et à l'I.C.P. Le contenu de la solution de périphyton a été filtré sous pression d'azote sur des filtres GFC, préalablement lavés à l'acide. Les résidus déposés sur les filtres ont été digérés dans une solution de HNO_3, HCl, H_2O_2 et HF, puis dosés au spectromètre de masse ICP-MS. Les concentrations en métaux dans le périphyton ont été exprimées en µg/g de poids sec de périphyton par litre. Les analyses de laboratoire ont été menées au Centre Saint-Laurent. Pour l'analyse des métaux dans le seston, le même protocole a été suivi. Les valeurs de concentrations en métaux dans le seston ont été exprimées en µg/g de poids sec de matière en suspension/litre d'eau filtrée. L'échantillonnage et les analyses de laboratoire ont été effectués par une équipe du Centre Saint-Laurent, aux mêmes sites et à la même période que les autres étapes de l'échantillonnage. Pour les différents échantillons (sédiments, mollusques, périphyton, seston et macrophytes), les analyses de métaux ont été contrôlées avec des échantillons certifiés (NRCC-MESS-1, Tort et Oyster tissue-NIST SRM 1566, Citrus leaves-NIST SRM 1572, Orchard leaves-NIST SRM 1571, Apple leaves-NIST SRM 1515, et Peach leaves-NIST SRM 1547).

Méthode statistique:

Pour tenter d'expliquer les concentrations en métaux observées chez les gastéropodes, nous avons élaboré des modèles de régression multiple, en utilisant la concentration en métaux dans les gastéropodes comme variable dépendante.

Les variables indépendantes comportaient tout d'abord les concentrations en métaux dans les macrophytes, dont six espèces ont été considérées: *Vallisneria americana, Elocea canadensis, Myriophyllum spicatum, Potamogeton richardsonii, Heteranthera dubia*, et une espèce d'algue filamenteuse, *Cladophora sp*. Ces variables ont été considérées dans notre recherche en raison de leur association avec les gastéropodes phytophiles, qui utilisent les macrophytes comme substrat et probablement comme nourriture (Vincent et al., 1991; Aldridge in Wilbur, 1983). Les concentrations en métaux dans le périphyton (épiphyton) pourraient a *priori* se montrer les plus explicatives, étant donné la place importante qu'occupent les algues dans l'alimentation des gastéropodes brouteurs (Aldridge in Wilbur, 1983). Le seston pourrait également servir de voie de transfert aux contaminants, par ingestion ou incorporation au niveau des branchies de métaux adsorbés ou absorbés aux particules en suspension. En effet, les gastéropodes prosobranches, filtrent l'eau à la fois pour respirer (branchies) et pour se nourrir (Tashiro et Coman, 1982; Aldridge in Wilbur, 1983).

Les concentrations en métaux dans les macrophytes, le seston et le périphyton ont été regroupées sous l'appellation de "facteurs biotiques".

D'autres variables indépendantes, regroupées sous le nom de "facteurs abiotiques" et ayant déjà été considérées dans la littérature comme de bons "prédicteurs" de la bioaccumulation des métaux, ont été utilisées dans l'analyse de régression. Ces facteurs étaient: 1. Les concentrations en métaux biodisponibles, liés aux différentes fractions géochimiques des sédiments: métaux facilement échangeables (MF1), métaux liés aux carbonates ou spécifiquement adsorbés (MF2), métaux liés aux oxydes de fer et de manganèse (MF3) et les métaux liés à la matière organique et aux sulfures (MF4) (Langston, 1984; Tessier et al, 1984; Luoma et Davis, 1983); 2. Les concentrations en ions libres estimées à l'aide des équations de Tessier (Tessier, 1992 et Tessier et al, 1993). Elles sont considérées comme étant les concentrations en métaux à l'interface eau-sédiments, et sont fonction des métaux liés aux différentes phases géochimiques des sédiments oxiques (Tessier, 1992; Tessier et al, 1993; Amyot et al, soumis 1993). Les métaux pour lesquels nous avons pu estimer la concentration de l'ion libre étaient Cu, Ni, Pb, Zn et Cd. Les concentrations en ions libres semblent être le facteur le plus important pour le contrôle de l'assimilation d'un métal par les invertébrés (Luoma, 1983); 3. Les concentrations en carbone organique dissous (C.O.D.), considérées comme étant capables de complexer les ions des métaux traces (Neubecker et Allen, 1983; Luoma et Bryan, 1982); 4. Les concentrations en calcium dissous ($[Ca^{2+}]$) étant reconnues pour compétitionner avec d'autres éléments divalents aux sites de fixation cellulaire des membranes biologiques (Luoma, 1983); 5. Les concentrations en carbone organique dans les sédiments, étant connues pour influencer la biodisponibilité des métaux (Tessier et Campbell, 1990; Tessier et al, 1993); 6. La granulométrie des sédiments, pouvant influencer la biodisponibilité des métaux. En effet, les sédiments plus fins adsorbent de plus grandes quantités de contaminants que les sédiments plus grossiers (Salomons et Forstner, 1984); 7. Finalement, l'acidité ou la concentration en ion hydrogène ($[H^+]$) pouvant affecter la spéciation d'un métal en solution, sa biodisponibilité dans les sédiments et la sensibilité des membranes biologiques (Luoma, 1983; Campbell et Stokes, 1985).

En plus des facteurs biotiques et abiotiques, deux variables muettes (dummy variables) ont été intégrées dans l'analyse de régression, de façon à pouvoir tester l'effet de la taille des individus (juvéniles inférieurs à 6 mm, et adultes supérieurs à 6 mm) et de la période d'échantillonnage (juillet et août).

La procédure de régression multiple impliquant la normalité des données, chaque variable a subit une transformation logarithmique de façon à normaliser les données et homogénéiser la variance (Smock 1983, Kelly 1988), puis été testée à l'aide d'un test de Kolmogorov-Smirnov (Neter et al. 1990).

RÉSULTATS ET DISCUSSION

Tableau 1. Gradients des concentrations en métaux (µg/g poids sec) chez *Bithynia tentaculata* du Lac St-Louis.

Métaux	Étendue concentrations µg/g poids sec	Concentrations moyennes adultes	Concentrations moyennes juvéniles	Stations les plus contaminés	Stations les moins contaminés
Cadmium	0.28-2.56	1.06	0.94	5,6,10	3,8
Chrome	1.47-9.98	3.07	4.50	5,6,12	3,7,8
Cuivre	89.21-485.52	264.91	135.13	2,5,10	7,8,11
Fer	334.05-534.74	1204.37	1959.87	4,5,6	3,8
Manganèse	62.19-991.65	292.67	398.34	4,6,11	2,3
Nickel	0.62-991.65	2.96	3.48	2,3,4	7,9,10
Plomb	0.62-5.51	2.45	3.09	4,6,9	3,8,10
Zinc	143.86-529.64	326.17	226.32	6,10	7,8

Le tableau 1 résume les concentrations en métaux observées chez les gastéropodes. On remarque que le fer, le manganèse et le zinc sont les métaux retrouvés en plus grande quantité. Les stations où les concentrations en métaux dans les gastéropodes sont les plus fortes sont les stations 5,6 et 10. À l'opposé, les organismes des stations 3, 7 et 8 sont les moins contaminés. Les concentrations moyennes sont plus fortes chez les juvéniles dans le cas du Cr, du Fe, du Mn, du Ni et du Pb. Au contraire, les concentrations moyennes en Cd, Cu et Zn sont plus fortes chez les adultes.

Les organismes peuvent accumuler des métaux en solution dans l'eau, sous forme d'ions, ou par voie trophique, par ingestion de métaux absorbés ou adsorbés aux particules alimentaires. L'importance de ces sources dépend des concentrations qui sont biodisponible et des quantités entrant en contact avec l'organisme. L'assimilation par les organismes peut être influencée par des facteurs physico-chimiques de l'environnement (Luoma et Bryan 1982; Luoma 1983).

Nous avons tenté de créer des modèles de régression multiple pour prédire les concentrations en métaux traces Cd, Cu, Cr, Ni, Pb et Zn dans les gastéropodes en incluant à la fois des variables biotiques et abiotiques. De plus, deux autres métaux, le Fe et le Mn ont subi le même traitement statistique, pour l'élaboration de modèle de prédiction, bien qu'ils ne soient pas considérés comme étant toxiques aux niveaux habituellement rencontrés. Le tableau 2 montre les modèles pour chacun des métaux pour lesquels des relations significatives ont été trouvées.

Les modèles:

Le modèle du Cadmium qui explique 77% de la variance observée, inclut comme variables indépendantes la concentration en Cd dans les macrophytes *Myriophyllum spicatum* et la concentration en ions libres Cd^{2+}. Une étude menée sur un ensemble d'invertébrés benthiques par van Hattum et al. (1991) n'a pu établir de modèle de prédiction de concentrations en Cd chez les mollusques (bivalves et gastéropodes), bien qu'ils aient pris en considération plusieurs biotiques et abiotiques. Tessier et al. (1993) et Luoma (1983) ont par contre démontré que les concentrations en ion libre Cd^{2+} semblent être le facteur le plus important pour le contrôle de l'assimilation du métal par les mollusques unionides et d'autres invertébrés aquatiques. Tessier et al. (1993) sont arrivés à expliquer 82% de la variance observée des concentrations en Cd chez le bivalve *Anodonta grandis*, seulement à l'aide de la concentration en ion libre (estimée par la formule de Tessier et al. 1993). Une autre étude, menée parallèlement à la nôtre, et utilisant l'amphipode *Gammarus fasciatus* comme organisme cible, a développé un modèle dont l'ion libre ainsi que trois facteurs physico-chimiques (H^+, Ca^{2+} et COD), sont les variables explicatives (R^2=0,68) (Amyot et al. 1993 soumis). La figure 3, illustrant la régression linéaire entre les valeurs prédites et observées de concentrations en Cd chez *Bithynia tentaculata*, montre qu'il n'y a pas d'effet de taille (juvéniles ou adultes) et de périodes d'échantillonnage (juillet ou août).

Tableau 2. Variable indépendantes retenues dans les équations de régression multiple pour la prédiction du logarithme de la contamination en métaux (µg/g poids sec), des gastéropodes Bithynia tentaculata en fonction des facteurs biotiques et abiotiques de leur environnement.

Métaux	Variables indépendantes	Coefficients de régression	R2	F	p(F)	N
Cadmium	ion libre Cd++	0.11	0.77	27.43	<0.0001	19
	Myriophyllum spicatum	0.66				
	Constante	0.53				
Chrome	Potomogeton richardsonili	0.44	0.88	41.45	<0.0001	14
	Taille	-0.12				
	Constante	0.68				
Fer	Vallisneria americana	0.40	0.65	12.31	<0.0001	31
	Calcium	-1.63E-4				
	Période	-0.2				
	Taille	-0.24				
	Constante	2.60				
Manganèse	Potomogeton richardsonili	0.86	0.94	49.42	<0.0001	14
	Période	-0.34				
	Taille	-0.13				
	Constante	0.66				
Zinc	Zn-F3	0.19	0.55	13.14	<0.0001	36
	% carbone organique	-0.20				
	Taille	0.18				
	Constante	2.03				

Les variables explicatives du modèle du chrome sont les concentrations en Cr dans les macrophytes de l'espèce *Potamogeton richardsonili* et la taille des gastéropodes. Il semble donc que les macrophytes soient un vecteur important de l'assimilation du chrome par les gastéropodes. Le modèle du chrome permet d'expliquer 88% de la variance observée. Bien que la figure 4 ne montre pas clairement que les individus juvéniles (taille inférieure à 6mm) ont tendance à être plus contaminés que les individus adultes (taille supérieure ou égale à 6mm), la taille est une variable significative dans le modèle. Cette observation semble appuyer les études de Boyden (1974), qui avait observé des concentrations métalliques plus élevées chez les mollusques marins juvéniles. De façon générale, cette relation allométrique s'avérerait vrai pour tous les métaux, bien que la relation liant le contenu en métal (µg) et le poids sec de l'individu (g) diffère d'une espèce et d'un métal à l'autre.

Dans le cas du fer, le modèle développé comprend les concentrations en fer dans les macrophytes *Vallisneria americana* (voie trophique), la concentration en calcium dissous (Ca^{2+}), ainsi qu'un effet marqué de la période d'échantillonnage et de la taille des organismes. Ce modèle explique 65% de la variance observée des concentrations sont supérieures au mois d'août, et que les individus de petite taille sont encore une fois plus contaminés que les adultes. La relation inverse observée pour le calcium dissous, concorde avec la théorie qui donne au calcium un rôle d'inhibiteur de l'assimilation des métaux chez les organismes aquatiques (surtout les poissons) (Luoma 1993). Forstner et Wittman (1981) et Salomons et Forstner (1984) font état de la complexation des métaux lourds avec les carbonates les rendant ainsi moins disponibles. De plus, les ions calcium sont reconnus pour faire compétition aux ions métalliques sur les sites de fixation des tissus biologiques. L'étendue des variations de la concentration en Fe dans les gastéropodes, au mois de juillet et au mois d'août est similaire sauf pour quelques stations. Ceci peut dépendre de plusieurs facteurs, difficilement identifiables. Selon Forstner et Wittmann (1981), l'activité biologique, le métabolisme, et la biomasse des organismes changent au cours d'une même saison, et influencent ainsi la bioaccumulation des métaux. Le modèle du Fe n'est pas parfaitement ajusté pour prédire les concentrations inférieures à 3 µg/g poids sec.

Le modèle du manganèse explique 94% de la variance observée des concentrations en Mn dans les organismes et comporte comme variables explicatives les concentrations en manganèse dans les macrophytes *Potamogeton richardsonii*, et les effets de taille et de période d'échantillonnage (figure 6). Comme dans le cas du fer, les concentrations en manganèse sont plus fortes chez les individus juvéniles à chaque période d'échantillonnage mais diffèrent entre les deux périodes d'échantillonnage (plus élevées au mois d'août qu'au mois de juillet).

La concentration en zinc dans la fraction 3 des sédiments (métaux liés aux oxydes de fer et de manganèse), le pourcentage de carbone organique dans les sédiments et la taille des organismes sont les variables explicatives du modèle prédictif de la concentration en zinc dans les gastéropodes. Le zinc est un métal essentiel qui semble être régulé par de nombreux organismes aquatiques (Timmermans et al., 1991); ceci expliquerait la relation moins forte établie entre les concentrations en zinc des organismes et celles de leur environnement. Une étude de van Hattum et al (1991), a développé un modèle prédictif du zinc dans les mollusques; ce modèle comprenait les concentrations en zinc dans les sédiments et dans l'eau, et n'expliquait que 26% de la variance observée. La matière organique dans les sédiments est en relation inverse avec la concentration en zinc dans les organismes. Contrairement aux modèles du chrome, du fer et du manganèse, et contrairement aux conclusions de Boyden (1974) et Watling et Watling (1976), les concentrations en zinc observées dans notre étude sont plus élevées chez les adultes que chez les juvéniles (figure 7).

Aucune relation n'a pu être établie pour le Cu, le Ni et le Pb. D'autres cas semblables sont rapportés dans la littérature. Luoma et Bryan (1982) ont tenté de développer des modèles pour la prédiction de concentrations pour plusieurs métaux chez un bivalve *Scrobicularia plana*, en incluant des variables physico-chimiques, ainsi que des données de concentrations en métaux dans différentes fractions de sédiments et dans une algue *Fucus vesiculosus*. Ils n'ont pas pu établir de modèles de prédiction satisfaisante pour le cuivre et le plomb. Également, Newman et McIntosh (1982) n'ont pas pu établir de bonnes corrélations entre les concentrations en Pb chez deux espèces de gastéropodes: *Campeloma decisum* (prosobranche) et *Helisoma trivolvis* (pulmoné), et celles de l'eau interstitielle, des fractions géochimiques des sédiments, de l'eau, du périphyton (aufwuchs) et d'une espèce de macrophyte *Cabomba sp*.

Validation des modèles développés:

De façon à voir rapidement si les modèles élaborés pouvaient s'appliquer à d'autres espèces de gastéropodes, nous avons appliqué nos modèles de régressions linéaires pour prédire les concentrations en métaux chez d'autres espèces de gastéropodes, pulmonés et prosobranches.

Les figures 8 et 13 montrent bien que le modèle du Cd, développé avec *Bithynia tentaculata*, sous-estime les concentrations en Cd retrouvées chez les pulmonés et les autres prosobranches. Le modèle du chrome, par contre, semble s'appliquer relativement bien aux pulmonés (figure 14), sauf pour *Stagnicola edodes*, et surestime légèrement les concentrations en Cr des prosobranches (figure 9). Le modèle du fer quant à lui semble relativement applicable aux pulmonés (figure 15) et surestime légèrement les concentrations en fer des autres prosobranches (figure 10). Le modèle du manganèse sous-estime les concentrations en manganèse des prosobranches (figure 11), sauf pour *Pleurocera acuta*, et semble relativement bon pour la prédiction des concentrations en Mn dans les pulmonés, bien que les points soient plutôt dispersés (figure 16). Finalement, le modèle du zinc surestime beaucoup les concentrations en zinc observées, aussi bien chez les pulmonés (figure 17) que chez les prosobranches (figure 12), à l'exception de *Valvata sp.* Il est intéressant de noter qu'à l'exception du zinc, les concentrations en métaux chez les pulmonés pourraient être plus facilement prédites que chez les autres prosobranches, à l'aide des modèles développés pour *Bithynia tentaculata*. En effet, on aurait pu s'attendre a avoir une meilleure réponse aux modèles par les prosobranches, les pulmonés ayant une physiologie différente. Ces dissimilarités peuvent aussi être dues au fait que les différentes espèces n'ont pas été récoltées à toutes les stations. Une analyse comparative station par station sera réalisée pour affiner les comparaisons interspécifiques.

CONCLUSION

Tessier et al, (1993) considèrent que les modèles de prédiction des concentrations en métaux dans les organisme aquatiques doivent être un pré-requis à l'utilisation de ces animaux en tant que bioindicateur. Les organismes doivent également répondre aux critères de bon bioindicateur définit par Kelly (1988): 1. Les organismes devraient être capable d'accumuler des polluants à des concentrations relativement élevées, sans être létales; 2. Ils doivent être sédentaires de façon à être représentatifs du site d'échantillonnage; 3. Ces organismes doivent également être abondants, bien connus du point de vue taxonomique et facilement identifiables; 4. Ils doivent être disponibles toute l'année; 5. Ils doivent être de taille raisonnable pour faciliter les analyses; 6. Finalement, la biologie et l'écologie de ces animaux devraient être bien connues.

A *priori* avant même d'avoir commencé à développer des modèles de prédiction, les gastéropodes répondaient déjà à plusieurs de ces critères. A *posteriori*, nos résultats démontrent que ces organismes peuvent être utilisés pour le développement de modèles de prédiction de la bioaccumulation des métaux en fonction de la contamination de l'environnement. Toutefois, à l'étape actuelle de nos recherches, on doit considérer que l'application de ces modèles est encore limitée, et ce, pour plusieurs raisons: 1) des modèles n'ont pu être développés pour tous les métaux à l'étude; 2) le modèle du zinc semble peu applicable; 3) différentes espèces de macrophytes semblent être de bonne variables explicatives pour plusieurs métaux, suggérant l'importance de la voie trophique pour le transfert des métaux aux gastéropodes, mais certaines variables qui semblaient prometteuses n'ont pas apporté d'explications additionnelles aux modèles (seston, périphyton, ...). D'autres facteurs reliés à la biologie de l'espèce et à la période d'échantillonnage doivent également être considérés. La taille des individus semble avoir une certaine influence sur l'accumulation des métaux dans les organismes, les juvéniles étant toujours plus contaminés que les autres, à l'exception du Cd et du Zn. On observe également dans certains cas (Fe et Mn), des différences significatives entre les données de contamination du mois de juillet et du mois d'août.

Enfin, les modèles de prédiction élaborés à l'aide de *Bithynia tentaculata* ne s'appliquent pas précisément à d'autres espèces de gastéropodes pulmonés ou prosobranches, à l'exception du fer.

Avant d'être en mesure d'utiliser *Bithynia tentaculata* comme outil de surveillance environnementale, il est essentiel que les modèles de cette étude soient validés dans le cadre d'un autre projet. Une nouvelle campagne d'échantillonnage à d'autres stations sur le fleuve St-Laurent (Contrecoeur, Lac St-François, Bassin Laprairie, Lac St-Pierre et Lac St-Louis), a été réalisée en 1992 et permettra d'approfondir cette première étude.

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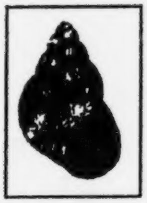

Figure 1. *Bithynia tentaculata.*

Site d'étude

Le lac Saint-Louis est un élargissement du fleuve Saint-Laurent situé en amont de Montréal, au confluent de deux masses d'eau: les eaux brunes originaires de la rivière des Outaouais au nord et les eaux vertes en provenance des Grands Lacs au sud. Ces deux masses d'eau possèdent différentes propriétés physico-chimiques. Ainsi, les eaux brunes situées au nord sont peu minéralisées et plus acides que les eaux vertes du sud (Sylvestre *et al.* 1992).

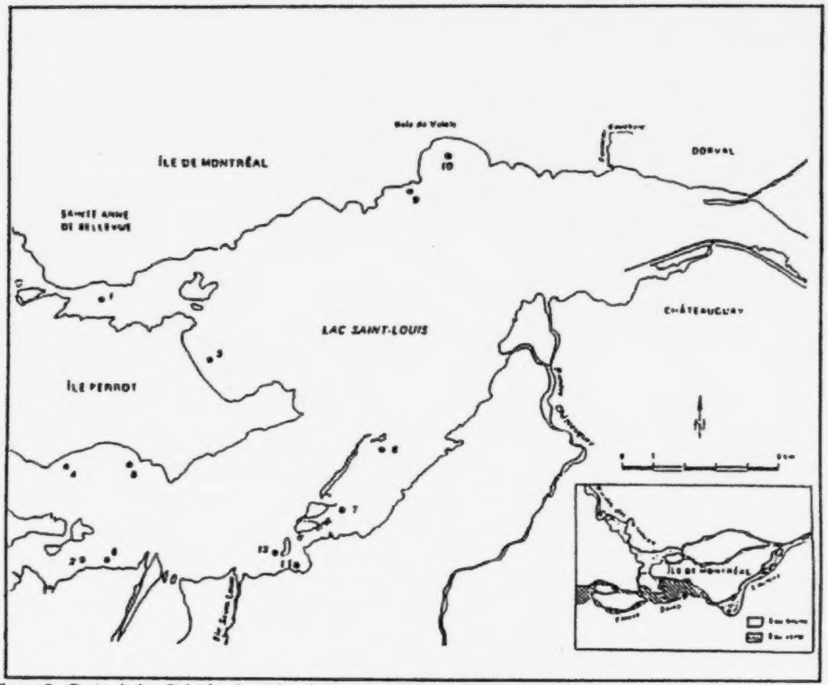
Baie de Valois
ÎLE DE MONTRÉAL
DORVAL
SAINTE ANNE DE BELLEVUE
10
9
1
LAC SAINT-LOUIS
CHÂTEAUGUAY
3
ÎLE PERROT
4
5
6
7
8
2
12
11
0
1
ÎLE DE MONTRÉAL

Figure 2. Carte du lac Saint-Louis, et localisation des stations d'échantillonnage.

Régressions linéaires entre les valeurs prédites et observées de concentrations en métaux chez *Bithynia tentaculata*.

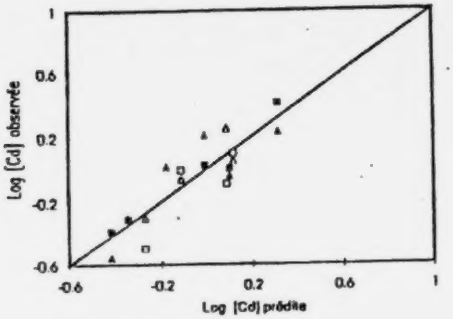

Figure 3. Cadmium

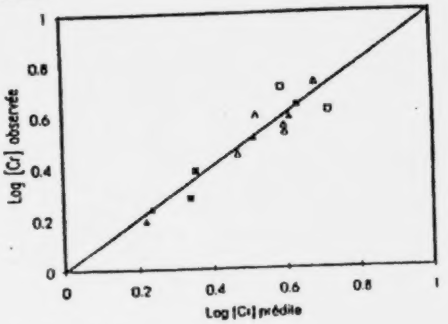

Figure 4. Chrome

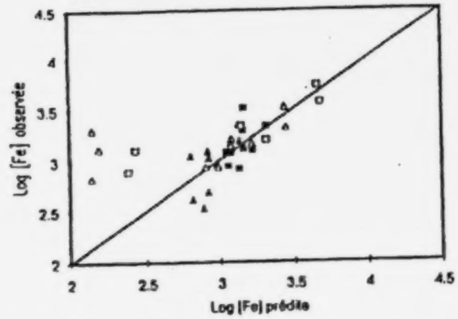

Figure 5. Fer

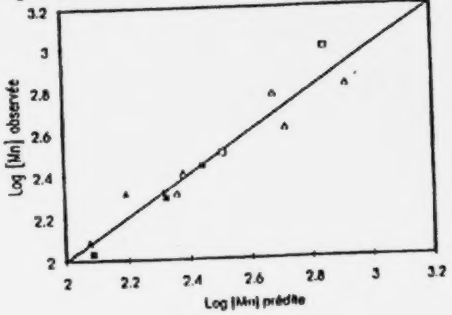

Figure 6. Manganèse

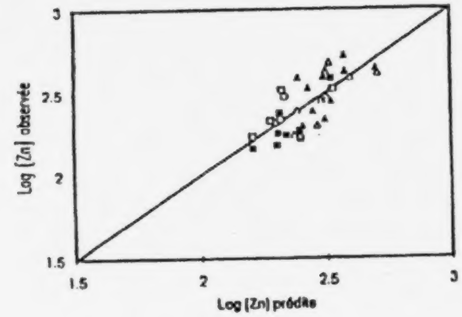

Figure 7. Zinc

■ Juvéniles - Juillet
▲ Adultes - Juillet
□ Juvéniles - Août
△ Adultes - Août

Régressions linéaires entre les valeurs prédites et observées de concentrations de métaux chez *Bithynia tentaculata* et d'autres espèces de gastéropodes prosobranches.

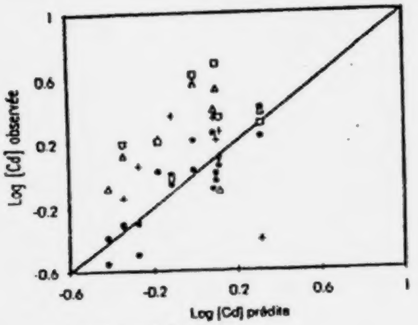

Figure 8. Cadmium

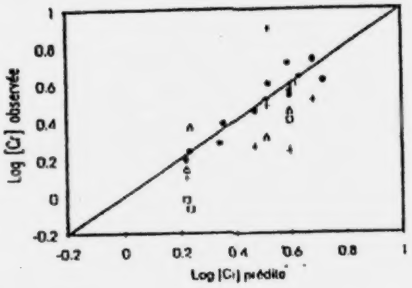

Figure 9. Chrome

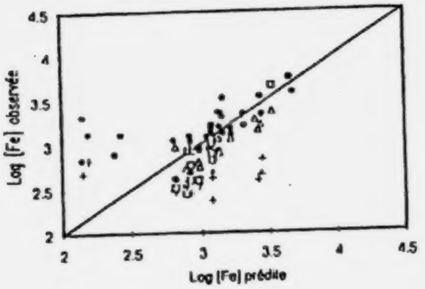

Figure 10. Fer

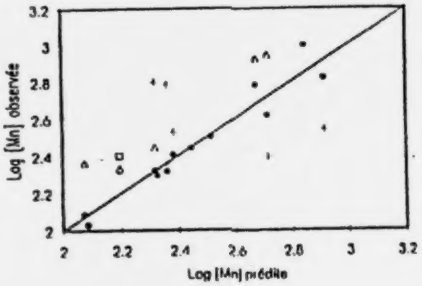

Figure 11. Manganèse

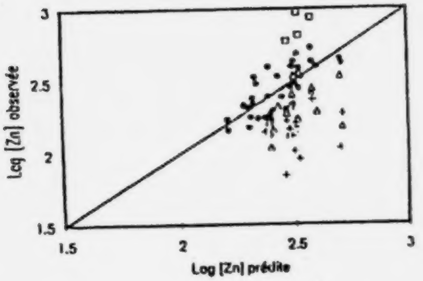

Figure 12. Zinc

•	Bithynia tentaculata
△	Amnicola limosa
+	Pleurocera acuta
□	Valvata sp.

Régressions linéaires entre les valeurs prédites et observées de concentrations en métaux chez *Bithynia tentaculata* et d'autres espèces de gastéropodes pulmonés.

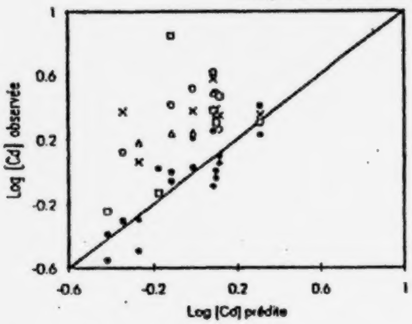

Figure 13. Cadmium

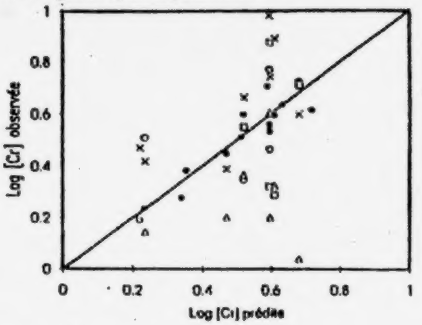

Figure 14. Chrome

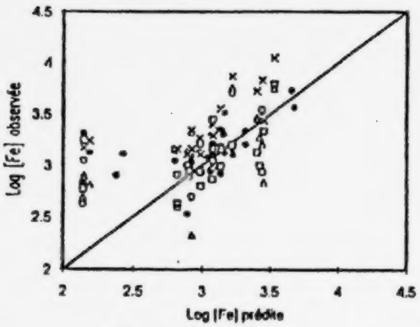

Figure 15. Fer

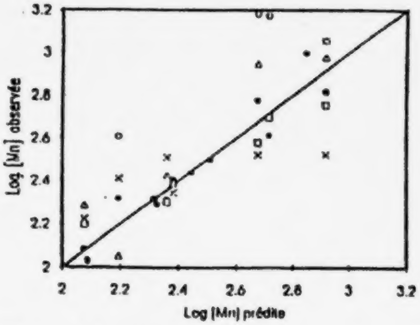

Figure 16. Manganèse

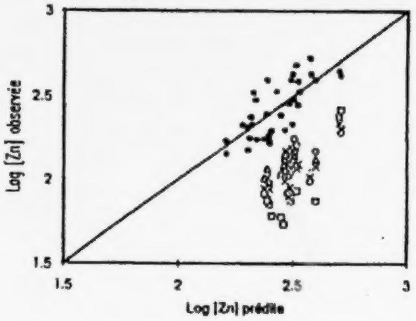

Figure 17. Zinc

● Bithynia tentaculata
○ Gyraulus sp.
□ Helisoma sp.
× Physa gyrina
△ Stagnicola elodes

SULPHIDE TOXICITY IN ANOXIC SEDIMENTS

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ABSTRACT

In oxic conditions, the sulphur in the sediment is bound and not bioavailable. However, under anoxia, sulphur is reduced, causing toxicity. A seasonal study of sediment from Stelco boatslip, Hamilton Harbour, showed that PAH (polynuclear aromatic hydrocarbon) contaminated sediment which is acutely toxic when anoxic (summer) is not toxic during the winter months when it is oxygenated (<10% mortality). When the same oxygenated sediment was forced to anoxia, it became toxic (98% mortality).

Organisms have different exposure levels to reduced sulphur. *Photobacterium phosphoreum*, in a direct contact bioassay shows a positive correlation between acute toxicity (99% inhibition) and sulphide concentrations (HVS: 32 μg/ mL, AVS: 173 μg/ mL) in the sediments. The ATP-TOX and Toxi-Chromotest bioassays, which use sediment extracts did not show a correlation. When sediment originally non-toxic in these bioassays (34%, 22% inhibition)(HVS: 10 μg/ mL, AVS: 92 μg/ mL) was spiked with H_2S (HVS: 147 μg/ mL, AVS: 404 μg/ mL), it was not immediately toxic to the organisms. However, when examined 22 days later, the sediment increased in toxicity (46%, 84% inhibition) even though the reduced sulphur concentrations had dropped (HVS: 19 μg/ mL, AVS: 146 μg/ mL). The hydrogen sulphide caused toxicity to these organisms indirectly by changing the sediment chemistry.

Key Words: hydrogen sulphide, coal tar, polynuclear aromatic hydrocarbon, *Photobacterium phosphoreum*, anoxic, volatile, *Hexagenia*, *Daphnia*

INTRODUCTION

Hamilton Harbour is located in a highly industrialized region at the west end of Lake Ontario (43°17'N, 79°50'W). The sediments of the harbour contain coal tar, with some polynuclear aromatic hydrocarbon (PAH) concentrations exceeding 200 μg/ g. In a 1990 study, Murphy et al. established a positive correlation to acute toxicity and coal tar concentrations.

In Hamilton Harbour, sediments containing high concentrations of coal tar also have elevated reduced sulphur concentrations. As the coal tar concentrations decrease, the reduced sulphur concentrations decrease accordingly. There are three distinct zones of toxicity, coal tar concentrations and reduced sulphur concentrations in the summer months; 1) highest in the boatslip regions near the steel industries, 2) moderate in the central, deepest portion of the harbour, or the depositional basin, and 3) lowest in the surrounding areas (Fig. 1).

The harbour is anoxic in the zones of high and moderate sulphur concentrations during the summer months. In the winter months even the boatslip sediment becomes oxic. Since previous studies on toxicity in Hamilton harbour have been conducted during the summer months, we have investigated the seasonal variability to determine if anoxia and hydrogen sulphide (H_2S) do contribute to toxicity. Non-toxic sediment was spiked with H_2S to induce toxicity.

Difficulties arise when measuring free H_2S concentrations in sediments (Brouwer and Murphy 1993). Bound H_2S does not purge readily from sediments for chemical quantification, although it will contribute to acute toxicity. We use the method of heat volatile sulphide (HVS) for measurement of bioavailable sulphide, unfortunately, at this time it is not known how much of the HVS is bioavailable due to the complexicity of sediment chemistry.

METHODS

Sample Collection:

Ekman grab samples were collected from Stelco Boatslip, Hamilton Harbour and a marsh near Long Point, on Lake Erie (41°58'N, 82°32'W) during different seasons of the year. The grab samples were homogenized and subsamples were frozen, freeze-dried or analyzed immediately (Fig. 2).

The sediment collected from Stelco Boatslip February 21, 1990 was subsampled and analyzed immediately. A portion of the sediment was purged for 24 hours with nitrogen gas, sealed then left for 45 days. The sediment, now anoxic, was analyzed as before.

Sediment from a north west control site in Hamilton Harbour; Carroll's Point, was used as control sediment in the *Daphnia* and *Hexagenia* bioassays. Long Point sediment was used as the uncontaminated control sediment for the microbial bioassays; subsamples were spiked with H_2S gas.

Bioassay Analyses:

Daphnia magna elutriates were extracted according to the protocol cited in Murphy et al. (1990). The samples were run in triplicate, further dilutions were made with dechlorinated water. Then *Daphnia*, 24 hours old were added to each sample. A dechlorinated water control was used to test the viability of the benthos. If there was less than an 80% survival of the *Daphnia* in the controls, the test was discarded and rerun. The duration of the test was 48 hours, the *Daphnia* were kept in an 25°C incubator. Survival was counted after 24 hours and again at the completion of the test, 48 hours.

Hexagenia bioassays were conducted as stated in Murphy et al. (1990). Dilutions were made by mixing "clean" non-toxic mud from the west end of Hamilton Harbour with the contaminated sediment. This site was chosen to ensure homogeneity of substrate in the tests. If there was less than an 80% survival rate in the controls, the tests were discarded and rerun.

Photobacterium phosphoreum bioassays were conducted on sediment according to the modified procedure of Brouwer et al (1990). Dilutions for EC_{50} calculations were made with "clean" sediment from Long Point.

The ATP-TOX method of Xu and Dutka (1987) was used on 10% dimethyl sulfoxide (DMSO), 10% methanol elutriates. Equal volumes of sediment and DMSO extractant were shaken vigorously by hand for 2 minutes. The homogenized mixture was then centrifuged for 20 mins at 10 000 rpm. The system uses a measurement of ATP as and indication of microbial growth. If, when compared to the control, a sample inhibits ATP production (i.e. growth), a toxic effect is assumed.

The Toxi-Chromotest method of Orgenics (1985) was used on 10% dimethyl sulfoxide (DMSO), 10% methanol elutriates, prepared as for the ATP-TOX bioassay. The colorimetric assay determines toxicity by detecting the inhibition of enzyme (beta galactosidase) synthesis by a mutant strain of *Escherichia coli*.

Chemical analyses:

The free hydrogen sulphide (H_2S), heat volatile sulphide (HVS) and acid volatile sulphide (AVS) analyses were conducted according to the procedure of Brouwer and Murphy (1993).

The water extracts used in the *Daphnia magna* bioassays were given to the National Water Quality Laboratory, Burlington, Ontario for analysis. Major ions and nutrients were analyzed by atomic absorption. Carbon was measured with an infrared analyzer. All procedures are outlined in environment Canada (1979).

Freeze-dried sediment was analyzed for polynuclear aromatic hydrocarbons (PAHs) on the GC/MS using the procedure outlined in Murphy et al. (1991).

RESULTS

Bioassay Results - Hamilton Harbour Sediment

The Oxygenated sediment samples collected February 21, 1990 showed no toxicity to the *Daphnia*. When this sediment was purged with nitrogen and left to incubate for 45 days, it became toxic to the organisms. The sediment collected in late summer was anoxic and showed acute toxicity to the *Daphnia*. Sediment was collected October 29 after the fall turnover. The water layers were stratified and the sediment was becoming oxygenated. The sediments were producing toxicity to 50% of the organisms; the borderline of toxicity. The sediment from February the following year confirmed earlier observations for February 1990 (Fig. 3).

Hexagenia and *Photobacterium phosphoreum* bioassays also showed a seasonal trend in toxicity. In both of these bioassays, the test organisms are in direct contact with the sediment (Fig. 4).

Chemistry Results - Hamilton Harbour Sediment

When the sediment collected February 21, 1990 was made anoxic, there were some increases in the concentrations of the metals iron (Fe), manganese (Mn) and copper (Cu). The metal zinc (Zn), was decreased significantly by the anoxia. Lead concentrations decreased significantly in sites 2 and 3 after anoxia. Copper values were significantly lower at the three other sampling dates and lead values were higher. Zinc values were much greater Feb. 21, 1991 than the previous year's samples (Table 1).

TABLE 1. Metal concentrations in Hamilton Harbour Aqueous Extracts

Summary of Water Quality Results: Metals

Daphnia magna Bioassay Elutriates:

	Fe mg/L	Mn mg/L	Cd µg/L	Co µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Hg µg/L
Feb. 21/90									
Site # 1	4.38	1.152	2.4	2.1	116.4	7.5	187.2	1071	0.12
Site # 2	3.09	0.609	3.3	1.8	110.7	7.5	316.8	1293	0.18
Site # 3	3.78	0.465	3.6	2.7	117.9	9.0	438.0	2091	0.24
Feb. 21/90 Anoxic									
Site # 1	10.11	3.750	2.1	2.7	140.4	19.5	214.2	122	1.11
Site # 2	3.42	2.307	<0.3	<1.5	171.3	7.5	75.9	243	0.84
Site # 3	10.56	1.533	2.1	1.8	240.6	19.2	244.2	1419	0.57
Sept.26/90									
Site # 1	6.34	0.251	6.3	2.3	28.6	5.4	564.0	1770	0.21
Site # 2	12.0	0.691	6.5	3.5	49.1	15.2	799.0	52	0.08
Site # 3	7.95	0.647	1.5	2.3	14.0	6.2	140.0	703	0.09
Oct. 29/90									
Site # 1	5.83	0.474	4.6	1.9	33.1	6.6	470.0	1740	0.54
Site # 2	9.43	1.370	4.9	3.3	48.1	15.0	452.0	2910	0.34
Site # 3	4.10	0.210	1.2	5.1	38.0	19.7	576.0	4590	0.73
Feb. 13/91									
Site # 1	3.76	0.922	3.2	2.2	27.9	9.2	428.0	2570	0.18
Site # 2	5.71	0.762	3.4	2.4	30.4	8.5	487.0	2640	0.33
Site # 3	111	1.730	1.0	1.4	15.4	10.3	50.7	398	0.08

No significant trends were observed in the major ion, carbon and nutrient concentrations at different seasons of the year. Although ammonium ion concentrations are high, at the pH of 6.7 of Hamilton Harbour sediments, less than 1% of the ammonium ions appear as un-ionized ammonia, the chemical from toxic to organisms (Thurston et al. 1979). The US EPA (1986) state that ammonia concentrations of 0.304 to 1.2 mg/ L are toxic to daphnids. (Table 2).

Table 2. Major Ion, Nutrient and Carbon Concentrations in Hamilton Harbour Aqueous Extracts

Summary of Water Quality Results: Major Ions

Daphnia magna Bioassay Elutriates

	Ca *	Mg *	Na *	K *	Cl *	SO_4 *	DOC *	DIC *	TKN *	NO_3 *	NH_4 *	TP **
Feb. 21/90												
Site # 1	60.0	15.0	25.8	13.5	31.0	13.6	21.4	78.9	16.40	0.08	17.21	298.5
Site # 2	45.0	13.6	27.4	12.8	29.7	23.0	23.6	81.0	15.89	0.16	16.40	279.8
Site # 3	55.0	16.7	29.0	14.6	33.6	11.9	33.4	99.8	23.64	0.12	22.96	321.6
Feb. 21/90												
Anoxic												
Site # 1	113.1	31.5	31.5	12.2	39.6	4.5	23.4	84.0	17.49	0.09	16.80	310.5
Site # 2	120.3	44.4	32.1	17.3	34.8	7.9	27.0	88.5	16.44	0.23	15.90	235.2
Site # 3	89.4	40.8	36.9	26.7	46.8	7.8	60.0	102.0	28.86	0.11	27.78	489.0
Sep. 26/90												
Site # 1	8.9	9.9	23.9	23.9	26.6	5.6	19.6	51.0	33.98	0.02	35.60	192.8
Site # 2	14.2	5.4	37.9	73.9	32.9	12.4	20.1	37.2	69.90	0.08	58.80	426.1
Site # 3	34.9	13.0	29.1	10.4	43.2	8.0	43.5	33.7	11.33	5.87	8.65	71.7
Oct. 29/90												
Site # 1	18.9	13.4	20.0	11.3	26.2	2.4	18.3	42.5	12.43	0.03	6.30	182.0
Site # 2	40.5	11.7	20.7	9.8	27.5	6.5	19.6	39.8	9.21	0.04	8.60	415.3
Site # 3	30.0	9.3	29.0	29.3	30.7	20.0	70.7	51.8	44.65	0.08	49.50	1.8
Feb. 13/91												
Site # 1	33.5	9.7	20.1	9.0	30.9	11.0	15.2	27.7	11.50	0.19	0.26	679.9
Site # 2	30.1	8.9	21.6	13.4	27.9	21.5	12.2	37.3	10.92	0.33	0.08	410.5
Site # 3	50.1	12.7	20.1	4.9	32.9	12.2	11.4	37.1	9.09	1.28	0.12	408.7

* (mg/ L)
** (μg/L)

PAH concentrations in the sediments from the three sites (average of 716.07 μg/ g) were above the 200 μg/ g biological cleanup recommendations proposed by Murphy et al. 1990. Site # 3, closest to the Stelco outfall pipe had the highest PAH concentrations (1426.72 μg/ g). *Daphnia* results from February 21, 1990, the same date that the samples were collected for PAH analysis showed more toxicity in site # 3 than the other two sites. The PAH concentrations did not change significantly during the study period (unpublished data).

Bioassay and Chemical Results - Long Point Sediment

The microtox bioassay showed a positive correlation between reduced sulphide concentrations and toxicity. Long Point sediment before spiking with H_2S (AVS 64 ppm, HVS 11 ppm) was non-toxic to the *Photobacterium phosphoreum*. After spiking the sediment with H_2S, the reduced sulphide concentrations had risen to 173 and 31.9 ppm for AVS and HVS respectively and the inhibition rose accordingly. The addition of 200 mg/ L iron to the spiked sediment reduced the toxicity, AVS and HVS concentrations.

Table 3: Toxicity to *Photobacterium phosphoreum* and the related sulphide concentrations

Hydrogen Sulphide Spiking Experiment - Long Point Sediment
Microtox Bioassay - *Photobacterium phosphoreum*

Sample	*Photobacterium* % inhibition (100% sample)	*Photobacterium* EC_{50} (100% Sample)	HVS (μg H_2S/ mL)	AVS (μg H_2S/ mL)
Control	0	N.A.	64	11
+ 50 mg/ L Fe	19	N.A.		
+ 200 mg/ L Fe	26	N.A.	14.8	54.5
H_2S Spiked	99	39	31.9	173.0
+ H_2S + 50 mg/L Fe	99	31	29.8	173.0
+ H_2S + 200 mg/L Fe	74	52	14.8	127.2

N.A. Not Applicable

The Toxi-Chromotest and ATP-TOX did not show immediate toxicity to higher reduced sulphur concentrations. When the original Long Point sediment was analyzed, it had low sulphide concentrations (AVS 91.6 μg/ mL, HVS 9.5 μg/ mL) and low inhibition. After the sediment was spiked, the sulphide concentrations increased to 404 μg/ L for the AVS and 147 µg/ L for the HVS, well above the concentrations used in the microtox bioassay. However, the toxicity only slightly increased for the Toxi-Chromotest and decreased for the ATP-TOX. When the same extracts were analyzed 22 days later, the sulphide concentrations decreased to 146 µg/ L for the AVS and 19.3 µg/ L for the HVS. The toxicity increased to toxic levels, averaging at 83.6% for the Toxi-Chromotest and 47.3% for the ATP-TOX.

Table 4. ATP-TOX and Toxi-Chromotest Bioassay Results and the Related Sulphide Concentrations.

Hydrogen Sulphide Spiking Experiment - Long Point Sediment
ATP-TOX and Toxi-Chromotest Bioassays

Sample	ATP-TOX % inhibition (100% sample)	Toxi-Chromotest % inhibition (100% sample)	S^{2-} (µmol/L extract)	HVS ((µg H_2S/mL)	AVS (µg H_2S/ mL)
Control:					
Rep # 1	34.8	24.4	1.7	9.5	91.6
Rep # 2	33.7	18.4	1.7		
H_2S Spiked-2 days:					
Rep # 1	16.7	37.0	100.0	147.0	404
Rep # 2	20.5	40.0	45.0		
H_2S Spiked-22 days:					
Rep # 1	40.8	78.0	1.8	19.3	146
Rep # 2	53.9	89.1	2.0		

DISCUSSION

The *Daphnia* have a high survival rate in the Stelco Boatslip sediment collected February 21, 1990 because the sediment is oxygenated. The sulphur is converted to sulphate, a non-toxic form, in the presence of oxygen, or is bound and not bioavailable. When it was forced to anoxia, the sulphur was reduced to hydrogen sulphide, thus becoming bioavailable and toxic. In September, the sediment if still anoxic, however by October, the temperatures are cooler, the oxygen content in the sediments has increased and the sediments are becoming oxygenated. The toxicity has decreased to borderline values; it is 50% toxic rather than 98% exhibited in September. In February 1991, the sediment is again saturated with oxygen and not toxic. Organic concentrations did not change over the year of study, therefore were not a factor in the toxicity differences.

Hexagenia and *Photobacterium phosphoreum* bioassays also showed a seasonal change in toxicity due to the presence of sulphide in the sediment. The *Hexagenia* incubation chambers were aerated throughout the duration of the experiment, however the sulphide was not easily driven off and the toxicity persisted. Bound hydrogen sulphide is not easily purged and released from the substrate (unpublished data). Zehnder and Zinder (1980) quote a half life of 19 +/- 19 h for hydrogen sulfide oxidation. In Hamilton Harbour where the sediments in the boatslip contain 20-300 mg/ L HVS and AVS values up to 100 times greater, H_2S oxidation will not be quick enough to prevent mortality from exposure. The LC_{50} for *Hexagenia* ranges from 0.312 mg/ L of H_2S ion solution for 48 h exposure to 0.060 mg/ L for 12 days (Oseid and Smigh 1975).

Metal concentrations do not influence toxicity. The National Water Quality Guidelines (U.S. EPA 1986) quote water quality threshold limits of 6.5 µg/ L, 108 µg/ L, for copper and zinc at the hardness of 50 mg/ L as $CaCO_3$, found in Hamilton Harbour. Copper and zinc values exceed 100 µg/ L and 1000 µg/ L in February when oxygenated, however in September the values have decreased by an average of 82% and 66%, although the sediment is much more toxic. There is not a direct correlation evident between toxicity and copper and zinc concentrations.

Sulphur chemistry plays an important role in toxicity of sediments. Sediments high in coal tar in Hamilton Harbour have high sulphur concentrations (greater than 1% total sulfur in boatslip sediments). PAHs contribute to chronic toxicity to organisms and the reduced sulphide cause acute toxicity. In the winter months when the sediment is oxygenated, it is not toxic to benthos in the lab. If the sediment could be treated in situ to keep it oxygenated in the warmer months, the sulphide would not cause toxicity and the microbes would be able to break down much of the harmful organics in the sediment.

Similar seasonal differences in toxicity have been observed in sediment from St.Marys River (Murphy et al. 1993). The ATP-TOX and *Photobacterium phosphoreum* bioassays showed a toxic response in the summer when the sediments were anoxic and no mortality in the winter months when the sediment was oxygenated. The differences were attributed to sulphide toxicity. Sulphide concentrations in the St.Marys River sediments are in the range of 0.3 to 4 mg/ L HVS.

Borgmann and Norwood also observed a seasonal variation in toxicity to *Hyalella azteca* exposed to Hamilton Harbour sediments. Sediments from regions of moderate sulphide concentrations showed an increase in toxicity in the late summer months. Unfortunately, sediments were not collected and analyzed from the winter months. Although a mechanism was not clear in the paper and sulphide concentrations were not measured, it is quite possible that the toxicity was caused by the presence of reduced sulphide.

Different bioassays are sensitive to different factors, therefore a series of bioassays approach is the best way to assess the samples in a community approach. The microtox bioassay shows a good correlation to sulphide concentration in the sediment. The Toxi-Chromotest and ATP-TOX bioassays are sensitive to sulphur species in a different way. Sediment that had been spiked with H_2S was not immediately toxic, however it was toxic 22 days later. It seems that the presence of sulphide had caused the formation of other metal sulphide or polysulphide that were detected in these bioassays.

ACKNOWLEDGEMENTS

Mike Fox conducted the PAH analyses. Laurie Lockington assisted with the laboratory work. Yvon Desjardins, Ken Hill and Bruce Gray assisted with the collection of sediment samples.

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Fig. 1. PAH Concentrations in Hamilton Harbour Sediments

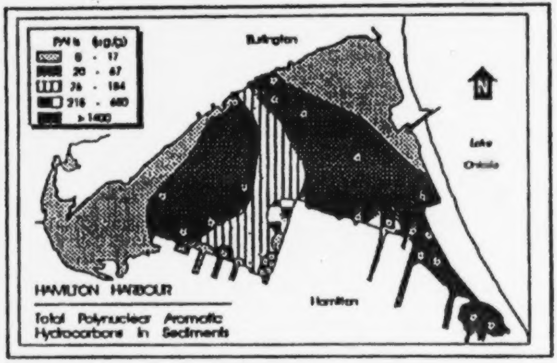
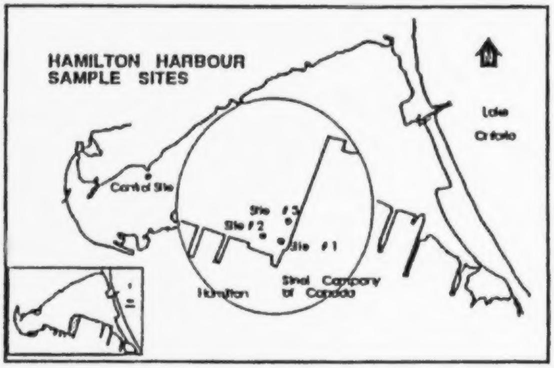

PAHs (µg/g)
0 - 17
20 - 67
76 - 184
216 - 680
> 1400
Burlington
N
Lake
Ontario
HAMILTON HARBOUR
Hamilton
Total Polynuclear Aromatic
Hydrocarbons in Sediments

Fig. 2. Sample Sites, Stelco Boatslip, Hamilton Harbour


HAMILTON HARBOUR
SAMPLE SITES
N
Lake
Ontario
Control Site
Site #3
Site #2
Site #1
Hamilton
Steel Company
of Canada

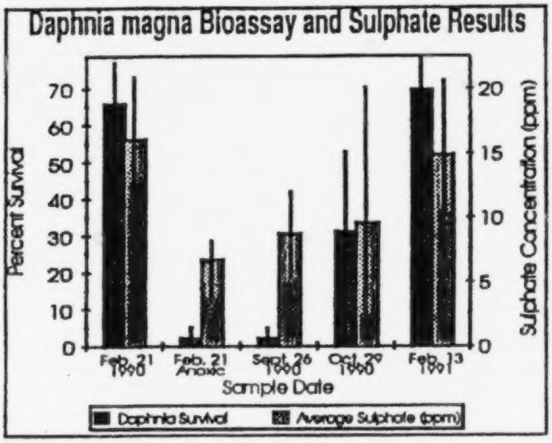


Fig. 3. Seasonal Trend in Toxicity as Indicated by Daphnia magna and Sulphate Concentrations of the Extracts.

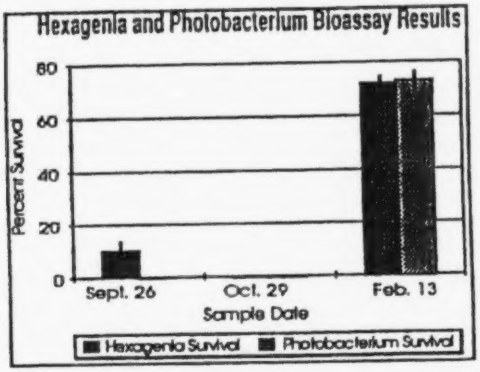


Fig. 4. Seasonal trend in toxicity as indicated by Hexagenia and Photobacterium phosphoreum

GENETICS AND APPLICATIONS OF THE TOD SYSTEM

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There is no doubt that a better understanding of the biochemical mechanisms by which microorganisms utilize aromatic hydrocarbons can lead to useful products and precesses. Improvements of the ability of microorganisms to degrade particular classes of environmental pollutants can also be made through molecular cloning and characterization of the genetic components involved in the biodegradative reactions and pathways. The *tod* system, which refers to the toluene degradation pathway by *Pseudomonas putida* F1 (PpF1), is an exemplification of these features and applications.

One of the unique characteristics of the tod pathway is the formation of a cis-toluene dihydrodiol as a result of oxygenation of the aromatic nucleus by an enzyme complex, termed toluene dioxygenase (Gibson et al. 1990). This initial reaction and the remaining 6 biochemical steps that lead to the formation of essentially harmless metabolites of the tricarboxylic acid cycle are shown in Fig. 1. The genes coding for all the enzymes and subunits have been cloned and sequenced. These structural genes are arranged in the order todFC1C2BADEGIH (Zylstra and Gibson 1989; Menn et al. 1991; this laboratory). Two regulatory genes, designated todS and todT, which control the tod pathway have also been found (this laboratory).

Toluene dioxygenase and its usefulness. The biochemistry and molecular genetics aspects of toluene dioxygenase (TD) have been well-studied and reviewed by Gibson and co-workers (1990). TD is a three-component enzyme system consisting of a flavoprotein reductase (todA), ferredoxin (todB) and an iron sulfur protein (ISP) made of two subunits (large todC1 and small todC2). For the enzymatic activity, NADH and oxygen are required. The formation of cis-toluene dihydrodiol from toluene necessitates the transfer of electrons from NADH to the terminal ISP oxygenase component (todC1C2) by the sequence shown in Fig. 1.

Besides toluene, a wide range of aromatic compounds, substituted of halogenated, can be oxidized by TD to yield pure hydroxylated products. TD can also function as a monooxygenase, eg. the oxidation of indane to indanol. Indigo formation from indole, and degradation of trichloroethylene (TCE), a common groundwater contaminant, are also be carried out by TD (for a review: Gibson et al. 1990).

Owing to the enantiomeric specificity of cis-toluene dihydrodiol, chemists such as Hudlicky and co-workers (1988) have used the dihydrodiol as a versatile chiral intermediate to synthesize products of pharmaceutical interest, eg. prostaglandins. Polymers such as polyphenylene can also be made using cis-benzene dihydrodiol as a precursor. The latter is a facile reaction product of TD oxidation of benzene. Otherwise, conventional chemical synthesis yields little product. Recently, TD has also been applied to the synthesis of 3-hydroxyphenylacetylene, a high-value chemical used in the manufacture of acetylene-terminated resins, a class of high-temperature polymers and adhesives (Williams et al. 1990).

Application of tod genes. The earlier determination of the tocC1C2BA genes which code for the initial TD enzyme complex of PpF1 (Zylstra and Gibson, 1989) paved way for the elucidation of homologous genes present in *Pseudomonas sp.* LB400 (Erickson and Mondello, 1992) and

P.pseudoalcaligenes KF707 (Taira et al. 1992). The latter two strains are capable of degrading polychlorinated biphenyls (PCB). Knowledge of the exact gene sequences of tod and the biphenyl (bph) system of KF707 has allowed Furukawa and co-workers (1993) to construct hybrid genes in order to expand the substrate range of the two systems and to study the gene components responsible for discrete substrate specificity of the TD and biphenyl dioxygenase. the replacements of bphA1A2 by todC1C2 in the bph system, for example, allowed KF707 to metabolize toluene. On the other hand, the tod system which normally does not use biphenyl as a sole carbon source will do so after acquiring the ring-cleavage bphD gene (coding for 2-hydroxy-6-oxo-6-phenylhexa-2,4-deinoate hydrolase) from the KF707 system.

In another elegant study and application, the tod pathway, due to its ability to metabolize benzene, has been combined with the mercury operon (merTPAB) genes to detoxify benzene pollutant from phenylmercury (Horn et al. in press). Recently, Wackett (1993) has combined the todC1C2BA genes with three genes from a *Pseudomonas* camphor system (camABC encoding the cytochrome $P450_{CAM}$) in order to dehalogenate the highly chlorinated pentachloroethane (PCE). In this recombinant strain, the cytochrome $P450_{CAM}$ first catalyzes the redctive dehalogenation of PCE to TCE which is then the substrate for the PpF1 TD. These data highlight the potential for construction of new metabolic pathways by gene recuitments to expand the range of detoxification of various contaminants and toxic pollutants.

Significance of regulatory genes. The importance of understanding how a degradative pathway is regulated cannot be overemphasized since specific genes of sets of genes (in an operon) can be activated or repressed depending on several factors. Factors influencing the performance of bacteria (in a bioremediation process) include optimal physicochemical conditions (eg. oxygen content, temperature, pH, etc.), availability and accessibility of substrates and nutrients. In PpF1, two regulatory genes, todS and todT, which function in a concerted manner to activate the tod pathway have been found (Lau et al. manuscript in preparation). The todS protein appears to have an oxygen-sensing domain in comparison with the sequence of a Rhizobium fixL protein which is known to sense oxygen but functions to regulate nitrogen fixation (Monson et al. 1992). Since the first step of toluene degradation requires oxygen, it will be instructive to find out how todS works and the molecular interactions which bring about the regulation.

The discovery of todS and todT is the first of its kind for the regulation of metabolism of aromatic compounds. These regulatory genes provide a new generation of DNA probes to screen other aromatic-degrading bacteria for the presence of similar or homologous genes. The rational of this approach, as opposed to the conventional gene probing work which is based on structural or catabolic genes per se, extends to the fact that even if isofunctional are present they can be regulated differently.

Biodegradation as a two-edged sword. One cuting edge of a biodegradative process is the mineralization of a pollutant; the other is the possibility of obtaining useful chemical intermediates for organic synthesis, as cited above. In connection with the latter, a special plasmid containing only todFC1C2BADEG genes has been constructed (this laboratory). This will allow the accumulation of 4-hydroxy-2-oxovalerate, a compound which is not commercially available, and a substrate for aldolase. Previously, mutant selection of a *P. putida* Biotype A strain has yielded commodity chemicals such as adipic acid and pyridine from the biotransformation of toluene (Hagedorn and

Maxwell, 1988). Undoubtedly, more applications of the tod pathway will unravel as we learn and understand more of the system.

Toxic intermediates in pathways. Although not the case in toluene degradation, accumulation of toxic intermediates can present a significant problem and indeed impedes the success of bioremediation. This demands a greater need for a detailed elucidation of the biochemical steps in any degradative pathway. If the source and identity of the toxic compounds are known it is likely that the problem can be rectified eg. by metabolic engineering.

In relation to a failing bioremediation process, physical contraints on electron flow and nutrient delivery can be an important factor. The dioxygenase pathway, which is representative in the metabolism of PCBs and polycyclic aromatic hydrocarbons, is illustrative of potential problems if the initial electron flow is channelled inappropriately.

As a concluding remark, and in keeping with the spirit of the ATW, perhaps it is appropriate to quote the following, seen aboard Queen Mary (off Long Beach, California) by one of us (P.L.) back in December 12, 1980. The illuminated billboard reads:

> This is the only planet in the universe
> blessed with an abundance of
> WATER.
> We must preserve it or perish.

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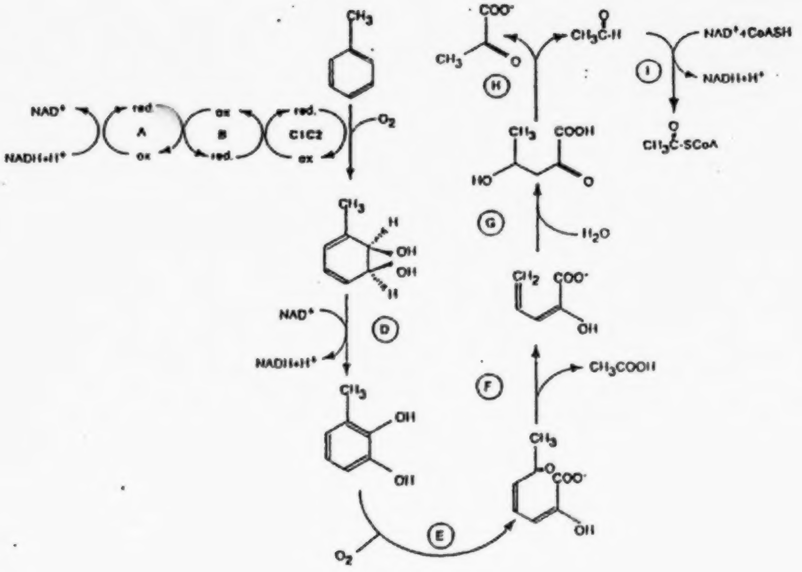
NAD⁺
NADH+H⁺
red.
ox
A
B
C1C2
O2
CH3
H
OH
D
E
F
G
H
I
CH3COOH
H2O
COO⁻
COOH
HO
CH3C-H
NAD⁺+CoASH
NADH+H⁺
CH3C-SCoA

Fig. 1. The *tod* pathway for the degradation of toluene in *Pseudomonas putida* F1. The enzymes, listed from A to I, are as follows:
toluene dioxygenase (todA, reductase; todB, ferredoxin; todC1, large subunit of iron sulfur protein (ISP); todC2, small subunit of ISP);
cis-toluene dihydrodiol dehydrogenase (todD);
3-methyl catechol 2,3-dioxygenase (todE);
2-hydorxy-6-oxohepta-2,4-dienoate hydrolase (todF);
2-oxo-pen-4-enoate hydratase (todG);
4-hydroxy-2-oxovalerate aldolase (todH);
acylating aldehyde dehydrogenase (todI).

EFFECTS OF 10-DAY GROWTH RETARDATION ON LONG-TERM SURVIVAL, EMERGENCE, AND FECUNDITY OF *CHIRONOMUS TENTANS*: POSSIBLE IMPLICATIONS FOR SEDIMENT TOXICITY ASSESSMENT.

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ABSTRACT

A life-cycle study was conducted with *Chironomus tentans* to assess the significance of growth retardation of 2nd-3rd instar larvae over a 10-d test period on long-term survival, emergence success, and fecundity. Larval growth was controlled by using 6 feeding levels ranging from 0.2 to 5.9 mg dry weight Tetrafin® fish food/day. Mean 10-d growth of *C. tentans* larvae displayed a strong linear relationship with measured feeding level. Growth at the six feeding levels were all statistically different ($p < 0.05$), yet mean 10-d survival was ≥ 88% in all cases. Cumulative successful emergence of adult *C. tentans* decreased significantly with decreasing feeding level and growth, ranging from 81% at the 5.9 mg/day feeding level to 0% at the 0.2 mg/day level. Times to emergence always increased with decreasing growth rates and effects were generally more pronounced for females than males.

Although fewer females successfully emerged at the 3.1 and 4.3 mg/day feeding levels, relative to the 5.9 mg/day level, ovipositing efficiency of those females did not appear to be adversely affected by their lower growth rates. Growth of second generation larvae did not appear to be affected by maternal feeding level and growth, but rather appeared to be solely correlated with their own feeding level.

INTRODUCTION

Chemical contamination of aquatic sediments is a serious problem in many of the heavily industrialized areas of the Great Lakes basin. These sediments contain a variety of inorganic and organic chemicals, frequently at concentrations that are toxic to members of the natural benthic community. It is important to biologically and chemically evaluate such sediments prior to dredging or remediation.

For biological evaluations of freshwater sediments, the non-biting midge, *Chironomus tentans*, has become a commonly used species in both field and laboratory tests. Tests with *C. tentans* have commonly started with 2nd-3rd instar larvae (10-14d old) and continued fo 10-14 d. Endpoints or measurements typically include survival and dry weight (or growth). The lethality endpoint of sediment toxicity tests is readily interpreted by the regulatory community as a serious adverse impact. However, the sublethal adverse endpoint of growth retardation is more problematic to interpret. It is not known for example, if a statistically significant retardation inlarval growth of one generation will cause biologically significant population reductions in subsequent generations. The objective of the study presented here was to evaluate the significance of retarded *C. tentans* growth upon its long-term survival, emergence success, and fecundity.

MATERIALS AND METHODS

Test system

Tests were initiated with *C. tentans* obtained from in-house cultures.

standard culturing methods were used (Call et al., 1992). Tests were run using a modification of a recently developed sediment testing intermittent renewal system (Benoit et al., 1993). The system used 300 mL beakers with 100 mL of silica sand per replicate. There were 12 replicate beakers per treatment. Beakers for a given treatment were placed within two glass aquaria, which received temperature-controlled dechlorinated municipal water (23°C) from a stainless steel headbox. The beakers had two 1.5-cm dia. opposing holes in their side-walls covered with 25-µm stainless steel screens to allow for water exchanged between the beakers and the aquarium. Water renewals ran for four 1-h periods each day.

10-Day Survival and Growth

Food was prepared by making a slurry of Tetrafin® goldfish food and deionized water. Measured concentrations were: 0.2, 1.3, 2.0, 3.1, 4.3, and 5.9 mg dry solids per mL. At test initiation, 10 larvae (10 to 11-d old) were placed in each test beaker. All beakers were fed 1.0 mL of the appropriate concentration of Tetrafin® food daily. After 10 d, 4 replicate beakers were randomly selected from each feeding level and removed for survival and dry weight determinations.

Emergence

On day 11 of the test, screen tops were placed on all remaining beakers to retain emerging adults. Emergence was recorded on a daily basis. At each treatment level, 4 of the remaining 8 beakers were designated for breeding adults and 4 for adult dry weight measurements.

Fecundity

Since the males at each feeding level emerged much sooner than the females, males from the UW-S stock culture were used daily to fertilize all females. A ratio of 3-4 males per female was maintained in each breeding chamber with no more than 2 females in any flask. Each morning, chambers were inspected for fresh egg masses.

Survival and Growth of Second Generation Larvae

Specially designed egg mass chambers were set up in the same test system described above. A 15-17 mm layer of sand was added to each chamber followed by of 0.2 mg Tetrafin®. One egg mass was added to each chamber the next day. The overlying water renewal system was not activated until 1 d after hatching. Food was added on the day that the majority of embryos hatched. At 10 d post-hatch, a 10-d growth test was initiated as described above. One set of 4 replicates were fed at the F_1 and at the 4.3 or 5.9 mg/day feeding level, respectively.

RESULTS

10-Day Survival and Growth

Mean 10-d growth (Y) of 2nd-3rd instar *C. tentans* larvae displayed a strong linear relationship with measured feeding level (X) best described by the equation $Y = -0.16 + 0.20X$ ($r^2 = 0.99$). Mean growth at each feeding level was significantly different from that at all other feeding levels (Table 1). There was no significant difference in mean survival among the six feeding levels. Dissolved oxygen concentrations generally decreased with increasing feeding level and only measurements at the 5.9 mg/day level were below 40% saturation.

Table 1. Mean 10-day survival and growth of 2nd-3rd instar *Chironomus tentans* larvae reared at various feeding levels.

FEEDING LEVEL (mg/day)	SURVIVAL (%)	GROWTH[(1)] (mg)
5.9	97.5	1.03 (0.02)*
4.3	90	0.74 (0.04)*
3.1	100	0.37 (0.04)*
2.0	100	0.27 (0.02)*
1.3	97.5	0.10 (0.02)*
0.2	87.5	-0.11 (0.02)*

(1) Growth = Final mean dry weight - initial dry weight.

* Growth was significantly different from that observed at all other feeding levels (α=0.05, $p<0.05$).

Emergence

Cumulative successful emergence of adult *C. tentans* increased notably with increasing feeding level. No adults ever emerged at the 0.2 and 1.3 mg/day levels, and only 8 (10%) organisms successfully emerged at both the 2.0 and 3.1 mg/ day levels. Cumulative successful emergence increased to 55 (69%) and 65 (81%) organisms at the 4.3 and 5.9 mg/ day levels, respectively (Table 2).

Time to emergence, measured relative to the day of larval hatching, was also significantly affected by feeding level. Generally, males emerged before females and the mean/median time to emergence increased with decreasing feeding level (Table 2).

The sex ratio of adult *C. tentans* was ≈ 1:1 at the 5.9 mg/ day feeding level, but increased to 7:1 (males: females) at 2.0 mg/day. This highly skewed sex ratio favouring males suggested that the energy requirement for successful female emergence was not met. The theory of limited energy availability for female emergence was further supported by the greater mean dry weight of successfully emerging females.

Fecundity

Egg masses were only collected from organisms at the three highest feeding levels, since no females were available for egg production at the lower three levels. The ovipositing success of the available females was high, ranging from 78 to 100%, with an overall success rate of 81%. Of the 22 egg masses produced, 29 (91%) were collected within 24 h of adult collection and fertilization.

Survival and Growth of Second Generation Larvae

Results for the 10-d growth study suggested that the initial F_1 larval feeding level had negligible effects on F_2 larval growth; growth appeared to be solely correlated with the F_2 feeding level.

Table 2. Mean and median time to successful emergence of *Chironomus tentans* at various feeding levels. All feeding levels started with 80 similar-sized 2nd-3rd instar larvae.

FEEDING LEVEL (mg/day)	SEX	n	MEAN TIME [±1 SD] to Emergence (Days Post-Hatch)	MEDIAN TIME TO EMERGENCE[1] (DAYS POST-HATCH)
5.9	M	32	26[4]	25^{a}
	F	30	33[4]	33^{A}
4.3	M	30	30[6]	28^{b}
	F	24	42[7]	44^{B}
3.1	M	5	33[9]	29^{b}
	F	3	48[7]	52^{B}
2.0	M	7	53[5]	55^{c}
	F	1	65[--]	65^{c}
0.2 & 1.3	M	0	NE[2]	-
	F	0	NE	-

[1] Times to emergence, within sex, followed by different letters are significantly different ($p<0.05$). Within a feeding level, capital letters denote a significant difference in median to emergence of males and females.

[2] NE = No emergence (no larvae survived to reach the pupal stage).

DISCUSSION

The primary objective of this study was to determine to long-term biological significance of reduced *C. tentans* larval growth over a 10-d sediment toxicity tests with *C. tentans*. A summary of the biological endpoints (Table 3) shows that larval survival was not affected over 10 d, even though growth was retarded by as much as 111% relative to the highest feeding level. Assuming that the growth retardations observed in this study can be equated with growth retardations often observed in sediment toxicity tests, this may suggest that if toxicants are present in sediments which are not acutely toxic, but which do result in retarded growth, long-term effects of biological significance could be expected.

The observed relationship between 10-d larval growth retardation (X) and reduction in adult emergence success (Y) could be described by the equation $Y = -5.6 + 1.2X$ ($r^2 - 0.95$). Although the data are somewhat limited, interpolation by linear regression indicated that a 50% reduction in emergence can be expected at a growth retardation of 45%. Therefore, if a 50% reduction in emergence is considered to be of biological/ecological consequence, then a growth retardation in a 10-d test with 2nd-3rd instar larvae of 45% could be considered a significant adverse effect. Whether emergence reductions

Table 3. Relationship between 10-day growth retardation of first generation *Chironomus tentans* larvae and adult emergence, fecundity, and growth of second generation (F_2) larvae.

Feeding level (mg/day)	10-d mean survival (%)	10-d Growth (mg)	10-d Growth Retardation[1] (%)	Emergence Success (%)	Emergence Reduction[2] (%)	Increase in Median Time to Emergence[2] (days)		Ovipositing Success (%)	F_2 Larval Growth (mg)
						M	F		
5.9	98	1.03	-	81	-	-	-	78	1.03[3]
4.3	90	0.74	28	69	15	3	11	8	1.01[3]
3.1	100	0.37	64	10	88	4	19	100	1.05[4]
2.0	100	0.27	74	10	88	30	32	-	-
1.3	98	0.10	90	0	100	-	-	-	-
0.2	88	0.11	111	0	100	-	-	-	-

(1) Relative to growth at the 5.9 mg/ day feeding level.
(2) Relative to emergence at the 5.9 mg/ day feeding level.
(3) When fed at a feeding level of 5.9 mg/ day.
(4) When fed at a feeding level of 4.3 mg/ day.

less than 50% (e.g. 30%) should be considered biologically/ ecologically significant, based upon this data, is subject to discussion. An ≈ 1:1 relationship was, however, observed between growth retardation and emergence reduction in the emergence reduction range of 5 to 50%. Based on these data, any observed growth retardation in a 10-d test with 2nd-3rd instar larvae between 10 and 50% can be expected to result in similar reductions in adult emergence success.

CONCLUSIONS

- Larval growth appears to be as sensitive, significant, and biologically important endpoint in 10-d sediment toxicity tests with *C. tentans*.
- Survival alone does not adequately describe potential long-term effects of sediment toxicity on *C. tentans* (assuming that the larval growth retardations reported here can be correlated with those caused by sediment-associated contaminants).
- Both survival and growth should be used as endpoints in 10-d sediment toxicity tests with *C. tentans*.
- Growth retardations of ≥64% did not affect 10-d larval survival, but resulted in significant reductions in adult emergence of 88-100%. This could have significant ecological consequences.
- Reduction in adult emergence (Y) under laboratory conditions can be predicted from growth retardation (X) by the equation $Y = -5.6 + 1.2X$ (in the growth range -0.11 to 1.03 mg dry weight per organism). Within the growth retardation range of 10 to 50%, this equates with a similar percent reduction in adult emergence.
- Assuming that at least a 50% reduction in adult emergence can be of biological/ecological significance, a growth retardation of ≥ 45% should be considered a significant adverse effect in a 10-d test with 2nd-3rd instar *C. tentans* larvae.

- Whether the observed relationships between growth retardation and long-term survival, emergence, and reproductive success holds when growth is altered as a result of a toxicant, not simply as a result of altering food availability, remains to be tested. This is required before the true significance of reduced larval growth in a 10-d *C. tentans* sediment toxicity test can be properly assessed.

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OIL BIODEGRADATION IN COLD SEAWATER - A MESOCOSM STUDY Robert Siron and Émilien Pelletier INRS-Océanologie, Centre Océanographique de Rimouski, 310 des Ursulines, Rimouski (Qc)1, G5L 3A1, Canada.

ABSTRACT

A group of five mesocosms (3.5 m^3 each) located at Pointe-au-Père (St.Lawrence Estuary), was used to study the behaviour of crude oil dispersed in cold and icy seawater (-1.8 to 5.5°C) under various environmental conditions. Experiments took place during Winter, Spring and Autumn and lasted from 2 weeks to 2 months. The bacterial response to the oil was assessed by recording the growth of total bacteria, viable heterotrophic bacteria and oil-degrading bacteria. The oil biodegradation was monitored by means of gas chromatography; some hydrocarbon ratios in both aliphatic and aromatic fractions of the oil served as biodegradation indexes. A "Combined Index of Biodegradation" (CIB) defined from the most significant hydrocarbon ratios was proposed to evaluate the overall biodegradation process. Mesocosms gave facilities to study the biodegradation in various parts of the aquatic environment, as the air-water interface (oil film and emulsions), the water column (dissolved components and dispersed oil droplets) and the settling oil residues. Interactions between bacterial and microalgal communities were also observed in mesocosms. The relevance of these experimental results to an oil spill in the St.Lawrence Estuary is discussed.

INTRODUCTION

Chemical and biological mechanisms governing the bacterial degradation of crude oil in marine environment are now well known (see among others, Lee & Levy (1989) and Atlas & Bartha (1992)). Although negligible in days following an oil spill, the biodegradation is the ultimate fate of oil over a long time. However, because numerous factors control this biotic process at sea, it is still difficult to set up descriptive models and, *a fortiori*, to predict the biodegradation rates (Mackay & McAuliffe, 1988). Several experimental and field programs have been conducted to study the oil biodegradation in cold and icy seawater, mainly in the Arctic (Bunch & Harland, 1976; Atlas et al., 1978). Some of these works examined the fate of chemically dispersed crude oil (Boehm et al., 1985; Siron et al., 1993) because oil dispersants are among efficient means to fight an oil spill at sea and have been worldwide used (API, 1986). In this respect, an experimental study has been developed over several years to investigate the fate and related effects of dispersed crude oil in cold and icy seawater. Emphasis was placed on interactions between oil and microorganisms (Delille & Siron, 1993; Siron et al., in press). We report here a summary of bacterial and chemical data addressing both the bacterial response to the oil and the related biodegradation of the oil spilled in St.Lawrence estuarine waters. This experimental approach was developed by using mesocosm facilities.

MATERIAL AND METHODS

Mesocosm experiments

Mesocosms located in the INRS laboratory at Pointe-au-Père (Québec) consists of a group of five tanks (V = 3.5 cm^3 each). Tanks were built with a stainless steel double-wall filled with a circulating coolant. Other specific devices (Fig. 1) offered the opportunity to study weathering processes under environmental conditions similar to those prevailing in arctic/subarctic climates (Pelletier et al., 1989). Particularly, an ice cover was maintained during winter experiments. Tanks set up in a cascade flow-through system (Fig. 1) simulated the natural dilution which progressively could occur from a spillage spot to surrounding waters. Experiments took place during Spring (16 days), Autumn (26 d) and Winter (13 d and 63 d). Physical-chemical properties of the seawater were specific of a subarctic estuarine environment (Table 1).

Contamination procedures

Several oil-dispersant combinations were tested; two light crude oils, the *Forties* (FOR) and the *Western Sweet Blend* (WSB) with similar properties (0.839 $g.ml^{-1}$; 9-10 cSt) and two dispersants, the *Corexit* 9527 (COR) and a surfactant mixture of AOT, Brij 92 and Brij 96 (ABB) were used with different natural-like procedures of contamination (Table 2):

a) Chemically dispersed crude oil added to tanks at different concentrations (static mode);
b) Chemically dispersed crude oil added in cascading tanks;
c) Oil adsorbed onto cotton strips and immersed in tank to simulate the release and weathering from an oiled substrate;
d) Untreated crude oil spilled at the water surface in static tank.

Bacterial counting

Techniques of bacterial enumeration had been detailed elsewhere (Delille & Siron, 1993; Siron et al., in press). Briefly, direct counts of total bacteria (TB) were monitored by epifluorescence microscopy using the acridine orange. Viable heterotrophic bacteria (VHB) were estimated by using: (i) the spread plate technique on marine agar (Difco, 2216 E medium); (ii) the Most Probable Number (MPN) procedure with the 2261E medium. Oil-degrading bacteria (ODB) were estimated by using either agar plates or the MPN procedure according to experiments, supplementing the basal mineral medium with crude oil as sole carbon source.

Oil analysis/biodegradation indexes

Seawater samples were regularly collected at 1m depth in mesocosms and dissolved/dispersed oil was extracted and quantified according to techniques previously detailed (Siron & Guisti, 1990; Siron et al., 1993). Both aliphatic and aromatic hydrocarbons were then separated on silica gel column and each hydrocarbon was identified and quantified by GC and GC-MS. The chemical part of this study was performed through the use and the development of chromatographic indexes, as specific markers of the oil biodegradation (Siron & Pelletier, 1993). Hydrocarbon ratios were calculated: (i) form oil droplets: C17/Pristane, C18/Phytane, Pristane/Phytane(Pr/Phy) and Σ [5 n-Alkanes]/Σ[5 isoprenoids], in the C14-18 boiling range (Alk/Iso); (ii) from the dissolved phase: Naphthalene/Phenanthrene, 2-Methylnaphthalene/1-Methylnaphthalene (2-MN/1-MN) and Methylnaphthalenes/total substituted naphthalenes (MN/Σ[MN+DMN+TMN]).

Table.1 Hydrobiological parameters recorded during mesocosm experiments.

EXPERIMENTS	AUTUMN	WINTER (long-term)	SPRING	WINTER (short-term)
Temperatures (°C):				
Seawater	1 ± 0.6	0 ± 1.5	4 ± 1.5	-1.6 ± 0.2
Ambient air	-16/5	-17/9	4/13	-16.7/-5.1
Ice coverage (days)	--	12+4+4	--	10
Salinity (°/$_{oo}$)	--	28	30	24
Diss. oxygen (% saturation)	143 ± 9	159 ± 24	146 ± 24	132 ± 11
pH	--	7.9/8.9	8.1/9.4	7.9/8.1
Suspended matter (mg.L^{-1})	1.1/3.2	1.2/5.3	2.3/12.2	1.6/3.8
Chlorophyll-a (μg.L^{-1})	--	<0.1/68.7	7.3/189.3	<0.1/0.8

TABLE 2. Oil contamination simulated in mesocosms.

EXPERIMENTS	TANK ASSIGNMENT[a]	CONTAMINANT[b] OIL/DISPERSANT	OIL CONC.[c] Max.	(mg/L^{-1}) Min.
Spring	T1: static	FOR/COR (10/1)	66.0	7.0
	T2: static	FOR/COR (10/1)	10.6	2.9
	T3: static	FOR/COR (10/1)	2.5	1.8
Autumn	T1: cascade (4%)	WSB/COR (15/1)	67.4	2.7
	T2: cascade (4%)	--	4.7	1.2
	T3: cascade (4%)	--	0.8	0.2
	T4: static	WSB/COR (15/1)	61.8	4.7
Winter (long-term)	T1: cascade (8%)	FOR/ABB (5/1)	44.6	0.3
	T2: cascade (8%)	--	5.6	0.3
	T3: static	FOR (no disp.)	0.9	0.5
	T4: static	FOR (adsorbed)	2.6	0.7
Winter (short-term)	T1: cascade (16%)	FOR/ABB (10/1)	4.6	1.7
	T2: cascade (16%)	--	2.0	1.6
	T3: cascade (16%)	--	1.5	0.7

a: Cascade flows are expressed in percent of tank volume per day;
b: Crude oils: Western Sweet Blend (WSB) and Forties (FOR); Dispersants: Corexit 9527 (COR) and surfactant mixture (ABB);\
c: Maximum and minimum oil concentrations measured at 1m depth.

In addition, a Combined Index of Biodegradation (CIB) defined from the most significant hydrocarbon ratios was calculated as follows:

$$CIB = [(Alk/Iso) + (Pr/Phy) + (2\text{-}MN/1\text{-}MN)] \times (100/S_{REF})$$

$$\text{where } S_{REF} = (Alk/Iso)_{REF} + (Pr/Phy)_{REF} + (2\text{-}MN/1\text{-}MN)_{REF}$$

S_{REF} is a reference value measured in fresh (undegraded) crude oil. As defined, the CIB is a relative index varying from 100 (fresh crude oil) to 0 (oil strongly degraded).

RESULTS AND DISCUSSION

Significance of contamination levels

The range of oil concentrations tested in mesocosms had been chosen to be representative of expected levels of contamination in various polluted environments (Table 2). Oil concentrations ranged from less than 1 mg.L^{-1} when crude oil was not treated by dispersant (i.e. chronic contamination in industrial areas of marinas), to more than 67 mg.L^{-1}, close to maximum concentrations measured in open sea under chemically dispersed oil slicks (API, 1986). Medium concentrations were obtained either by a dilution through the cascade system or by a release from oiled strips simulating the contamination of coastal waters after the shoreline was oil-impacted.

Heterotrophic growth

As expected, viable heterotrophic bacteria (VHB) were stimulated in oiled tanks. The bacterial enhancement depended on both the amount and the bioavailability of the crude oil. When the oil was chemically dispersed in the water column, the bacterial growth rate and the maximum density of bacteria reached in mesocosms generally increased with the oil amount (Fig. 2 & 3). However, in tanks where highest concentrations of oil were tested, a slight reduction of the bacterial growth was sometimes observed and could be due to the toxicity of some oil components to marine bacteria (Hodson et al., 1977; Fengerud et al., 1984; Siron & Pelletier, this volume). the release of adsorbed compounds from an oiled substrate also enhanced the growth of VHB as observed with the chemically dispersed oil, although the oil concentration was much lower (Fig. 3). In contrast, crude oil added alone did not significantly stimulate the VHB growth (Fig. 3). That was partly due to the low amount of crude oil accommodated in the water column (0.8. mg.L^{-1}) clearly had a stimulatory effect on the bacterial growth when the oil was chemically dispersed (Fig. 2). This result indicated the bioavailability of crude oil is an important factor which influences the bacterial activity. In all cases, the bacterial enhancement was observed within the very first days after the oil addition, without any lag phase. When dispersing agents are used, the crude oil is dispersed in very fine oil droplets with diameters lower than 10 μm in the case of efficient dispersions (Siron & Pelletier, 1993). As a result, the oil-water interface is strongly increased, allowing bacteria to attach to a substrate and to grow faster. The enhancement of heterotrophic activity by dispersed oil moreover could be partly due to the dispersant it-self (Parsons et al., 1984), either associated to the oil droplets or dissolved, in all cases increasing the load of easily assimilable carbon in the water column.

Bacterial adaptation

The stimulation of the heterotrophic activity by dispersed crude oils is clearly shown when comparing total bacteria (TB) and viable heterotrophic bacteria (VHB). In oiled tanks, VHB grew faster than TB, resulting in an increase of the VHB proportion over time. For example, bacterial counts differed from more than two orders of magnitude in natural seawater, but the difference was reduced to about one order of magnitude by the end of the spring experiment (Fig. 4). This "re-activation phase" of the natural community is usually associated to organic inputs (Delille & Bouvy, 1989). (In mesocosm experiments, the increase in VHB proportion was always observed following the oil addition. That could be considered therefore as an indication of the good adaptation of the indigenous bacterial community to the oil (Delille & Siron, 1993). A more specific adaptation to the oil is the increase of oil-degrading bacteria (ODB). As shown in figure 5, ODB increased over time and with the oil level tested. Maximum ODB densities however were measured at the end of experiments, contrasting with the VHB evolution. As a result, the proportion of ODB remained very low (< 1%) throughout the experiment, even in the most contaminated tanks. In this case, the density of ODB rather than the proportion was more convenient to indicate the specific adaptation to the oil. In this respect, ODB measurements are greatly depending on environmental factor (e.g. oligotrophic conditions or enriched seawater) and also are affected by growth medium and counting techniques (Walker & Colwell, 1976). Consequently, there is a lack of correlation between ODB measurements and contamination levels, as confirmed by the literature review (Siron et al., in press).

As expected regarding to the temperature-dependent bacterial metabolism, ODB grew faster in spring than in winter, following a shorter lag phase. Maximum densities reached in mesocosms however were similar for somewhat comparable oil concentrations (Fig. 5).

Oil biodegradation

It is well established now that nutrients are the major factors limiting the oil biodegradation at sea (Lee & Levy, 1989; Atlas & Bartha, 1992). In this respect, mesocosm experiments were conducted under non-limiting conditions (N and P salts were regularly supplied to maintain the same enrichment as measured in the natural seawater pumped from the St.Lawrence Estuary), in order to study some environmental factors that were suspected to greatly influence the bacterial activity in boreal/sub-boreal marine environments.

- Effects of the oil concentrations (spring and short-term winter experiments)

The decrease in some ratios of easily degradable hydrocarbons to more recalcitrant hydrocarbons gives a good evidence of the in-situ bacterial degradations, especially when combined with an experimental oil contamination (Madsen, 1991).

During mesocosm experiments ratios of linear to branched alkanes (Alk/Iso, C17/Iso, C17/Pr and C18/Phy) always displayed a similar decline. The Alk/Iso ratio that involves 10 alkanes for calculation proved to be more accurate to monitor the biodegradation of the aliphatic fraction (Fig. 6). In the aromatic fraction, changes in substituted naphthalene ratio are more subtle (Fig. 6); first, the increase of the MN/[MN+DMN+TMN] ratio just after the oil addition was likely due to the higher solubility of methylnaphthalenes. After a few days, the biodegradation of dissolved aromatic was revealed by the decrease of both 2-MN/1-MN and MN/[MN+DMN+TMN] ratios. The ratio involving methylnaphthalene isomers appears very convenient to survey the biodegradation of the aromatic fraction. The preferential degradation of the isomer 2-MN over the 1-MN could be explained by the mineralization pathways which involve different enzymatic mechanisms (Dean-Raymond & Bartha, 1975). Moreover, the significance of this ratio is reinforced by the fact that methylnaphthalenes are among the most toxic soluble hydrocarbons of crude oil (Siron & Pelletier, this volume).

In the short-term winter experiment conducted at sub-zero temperatures, the biodegradation was reduced, but was still detectable through Alk/Iso and Naph/Phe ratios (Fig. 7). In that case, the Naph/Phe ratio is more sensitive to feature the bacterial activity, with respect to the preferential mineralization of naphthalene by marine bacteria (Heitcamp & Cerniglia, 1987). Despites the low oil concentrations tested during this winter experiment, changes in biodegradation indexes reflected the oil toxicity to the bacterial community which was naturally stressed under extreme winter conditions; the higher the oil concentration, the slower the biodegradation (Fig. 7).

- Effects of a natural organic enrichment (Spring experiment)

The unexpected enhancement of the biodegradation in the least polluted tank, as revealed by the decrease of the Alk/Iso ratio (Fig. 6) could be related to a natural bloom of phytoplankton (up to 189 μg chl-a.L^{-1}), while microalgae were early inhibited in the two heavily oiled tanks (< 10 μg chl-a.L^{-1}, after one week exposure to oil). Growth measurements confirmed bacteria had benefited from rich and easily assimilable organic matter by the end of the experiment (Fig. 4), while phytoplankton was growing. The hydrocarbon degradation was confirmed through the decline of the Pr/Phy ratio, probably via a cometabolism process. The pristane molecule, indeed, is theoretically less recalcitrant to the biodegradation than phytane, with respect to the terminal groups (Rontani & Giusti, 1986). In addition to be a good marker of oil contamination in the marine environment (Siron & Giusti, 1990; Pelletier et al., 1991), the Pr/Phy index could be used therefore for ascertain the potential capability of microorganisms to degrade certain recalcitrant oil fractions (non linear hydrocarbons).

-Effects of the oil treatment (long-term winter experiment)

The biodegradation rate was slightly modified according to the oil treatment tested (Fig. 8). Actually, the main difference was observed between untreated and chemically dispersed crude oil. Dispersed oil was faster degraded, as revealed by Alk/Iso and 2-MN/1-MN ratios. Particularly noticeable is the fact that the biodegradation rate was somewhat comparable between dispersed and released oil (Fig. 8), revealing the capability of indigenous bacteria to degrade dissolved (released) aromatic hydrocarbons, as well as aliphatics (dispersed oil droplets). Finally, the biodegradation showed a similar progress over the second month of the experiment, whatever the initial treatment was. These results are in good agreement with the heterotrophic growth (Fig. 3). Some discrepancy however may be note between the response of oil-degrading bacteria (growth measurements; Fig. 5) and chemical data (oil biodegradation), confirming the difficulty to enumerate specific bacteria.

-Effects of the seawater temperature

The biodegradation rate measured in mesocosms was dependant on the seawater temperature, as shown when date from different experiments were plotted together (Fig. 9). This result was expected from bacterial responses to the oil. It is noteworthy that only a small increase of the seawater temperature seemed to be sufficient to enhance the oil biodegradation. The long-term winter experiment was characterized by icy conditions during the first twelve days (-0.9 ± 0.4°C) and then the water temperature gradually rose up to 3°C by the end of the experiment. The short-term winter experiment was conducted almost entirely under icy conditions (a thick ice cover formed early in tanks). Data from these experiments clearly showed the threshold of temperature to observe a significant biodegradation was around 0°C. This observation is supported by the fact that the bacterial community sampled in the St.Lawrence estuarine water in winter was a psychrotrophic rather than a truly psychrophilic community (Delille & Siron, 1993).

-Biodegradation at the air/water interface

Floating water-in-oil emulsions (the so-called "chocolate mousse") always showed typical unaltered chromatographic patterns (Fig. 10: A). In the same time, the 500 µm thickness surface microlayer was sampled in irridescent sheets by means of a metal sieve. The biodegradation of oil spread on the surface was found closely comparable to that measured in the water column at the same periods, regarding to the selective disappearance of n-alkanes to isoprenoids (Fig. 10: B). Severe weather conditions encountered over the winter experiment did not affect the biodegradation of the surface oil film. These experimental results confirm some field observations indicating that emulsified oil is very refractory to natural biodegradation (Boehm et al., 1982; Siron et al., 1991).

- Biodegradation of crude oil adsorbed on substrate (long-term winter experiment)

The biodegradation of crude (non dispersed) oil progressed faster in the water column (i.e. on crude oil adsorbed onto immersed cotton strips) than on the emerged wet part (2 cm above the surface) of the oiled sustrate (Fig. 110. In the other hand, the biodegradation was only slightly reduced at the air-water interface at the very end of the experiment, in agreement with that observed on dispersed oil. Oil-degrading marine bacteria likely find best conditions for degrading oil accommodated in the water column rather than on partly emerged substrate which is exposed to severe weather conditions in winter. One may assume that icy conditions are therefore of critical importance to determine the biodegradation of oil residues stranded on intertidal zones or trapped into ice-field of the St.Lawrence Estuary, as observed in Arctic (Atlas et al., 1978).

-Biodegradation of setling oil

Settling oil residues collected in sediment traps at the end of experiments were quite degraded, with most of the time a complete disappearance of light hydrocarbons which make difficult calculation of biodegradation indexes. The biodegradation always was more marked in trapped residues than in the water column, even after the short-term winter experiment where isoprenoids became prominent in traps (Fig. 10: C,D). That could be related again to bacterial enhancement by natural organic inputs via the sedimentation of microalgal cells (Siron et al., 1993). The small part of spilled oil that could reach the bottom of a coastal environment would be therefore very degraded when accumulated and mixed with natural organic matter at the water/sediment interface.

-"Combined Index of Biodegradation"/Summary

This index was developed to allow for the biodegradation of both the dispersed (aliphatic hydrocarbons) and the dissolved (mainly light aromatic) fractions at once. In this respect, the CIB could be helpful to evaluate the achievement of the oil biodegradation under natural conditions of weathering. It can be used also to compare the biodegradation progress on different crude oils or oil treatments. For example, all determining factors previously discussed are highlighted by calculating the CIB. Minimum values, reflecting maximum degradation, were found in the water column at the end of both long-term winter and spring experiments (CIB around 30-40), whereas maximum end-values (70-85) were measured in the short-term winter experiment (Fig. 12). Comparable values were obtained in the surface microlayer, the CIB decreasing from about 80 (2 weeks) to less than 40 after 2 months.

CONCLUSIONS

This study highlights the efficiency of mesocosms as experimental tools to determine the in-situ biodegradation of contaminants under various environmental conditions. the capability of the St.Lawrence Estuary bacterial community to adapt to an oil spill was demonstrated. Under favourable conditions, the biodegradation would significantly alter the oil within a few days. Moreover, in micro-environments where oil residues accumulate with organic matter, the oil biodegradation will be naturally enhanced. In the other hand, winter is confirmed to be the critical period for an oil spill occurring in boreal/sub-boreal environments because of the significant reduction of the biodegradation under icy conditions. In this respect, the use of chromatographic indexes appears as unequivocally means to ascertain the oil biodegradation. Some hydrocarbon ratios like n-alkanes to isoprenoids or n-alkenes to hopanes have been successfully used for many years to assess the effectiveness of bioremediation procedures (Ladousse & Tramier, 1991; Bragg et al., 1992). These indexes however are representative of the less toxic fraction of crude oils. The approach developed in mesocosms, by taking into account several oil fractions - including dissolved toxic aromatic hydrocarbons- would be helpful for example, in the assessment of the toxicity reduction during and after bioremediation or in the biotreatment monitoring of petroleum industry wastewaters, regarding to complex mixtures to be degraded under extreme environmental conditions.

ACKNOWLEDGMENTS

Authors with to thank M. Leclerc and C. Brochu for their technical assistance. This study was funded by the NSERC of Canada Through its strategic grand program (STRGP 036).

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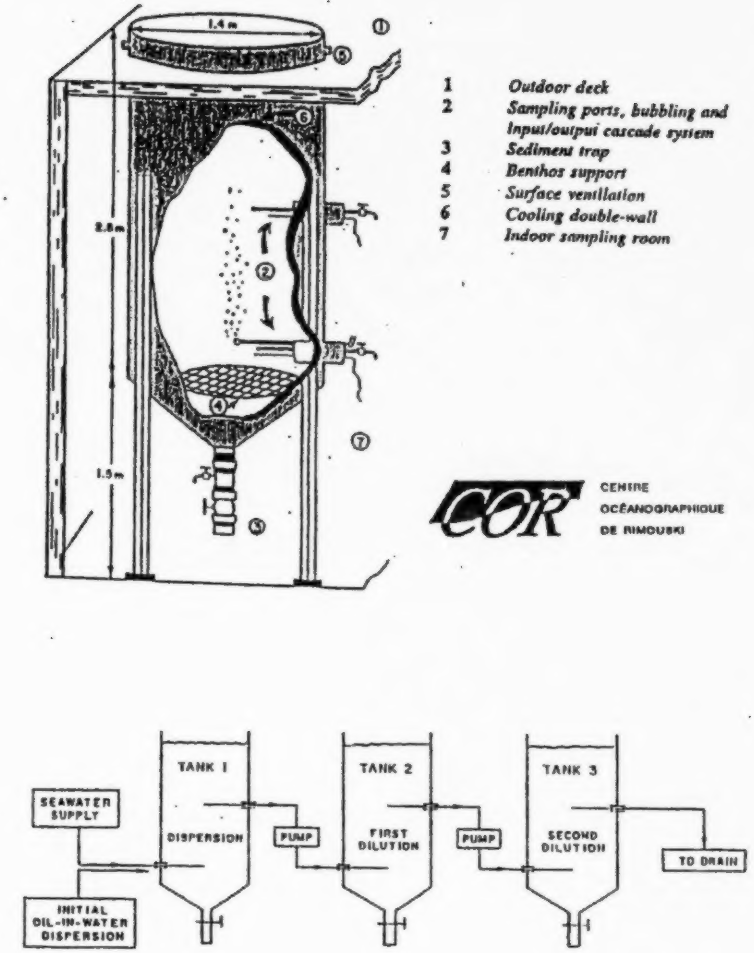
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3 Sediment trap
4 Benthos support
5 Surface ventilation
6 Cooling double-wall
7 Indoor sampling room
COR
CENTRE OCÉANOGRAPHIQUE DE RIMOUSKI
SEAWATER SUPPLY
INITIAL OIL-IN-WATER DISPERSION
TANK 1
DISPERSION
PUMP
TANK 2
FIRST DILUTION
PUMP
TANK 3
SECOND DILUTION
TO DRAIN

Figure 1. Mesocosms: specific devices and cascade mode operation set-up.

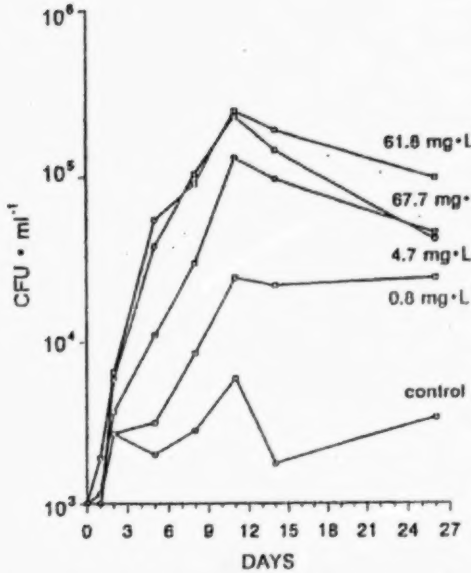

Fig.2 Autumn experiment: growth of viable heterotrophic bacteria in tanks contaminated with various concentrations of dispersed oil (maximum concentrations measured in each tank are indicated).

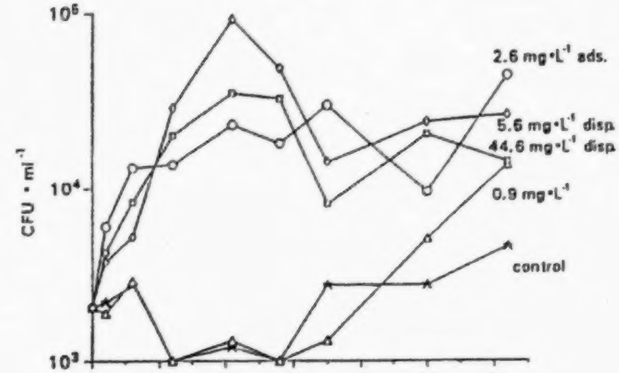

Fig.3 Winter (long-term) experiment: growth of viable heterotrophic bacteria in tanks contaminated with various oil concentrations and treatments (adsorbed, chemically dispersed and surface spilled crude oil).

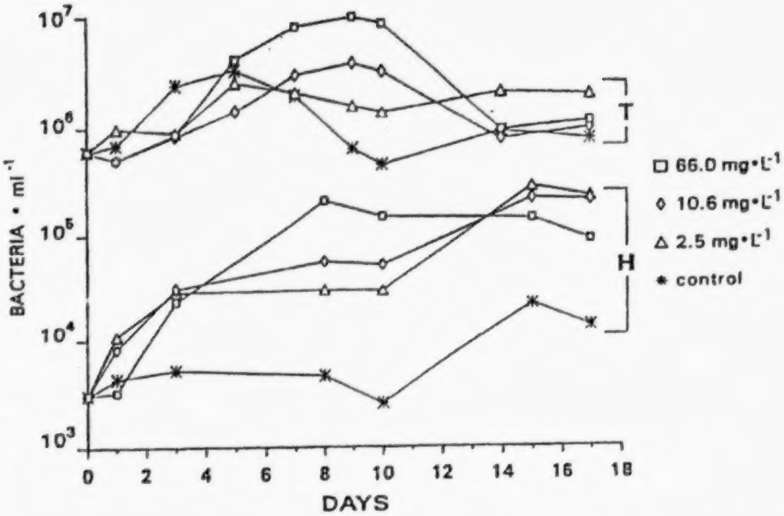

Fig.4 Spring experiment: growth of total bacteria (T) and viable heterotrophic bacteria (H) in tanks contaminated with various concentrations of dispersed oil (maximum concentrations are indicated).

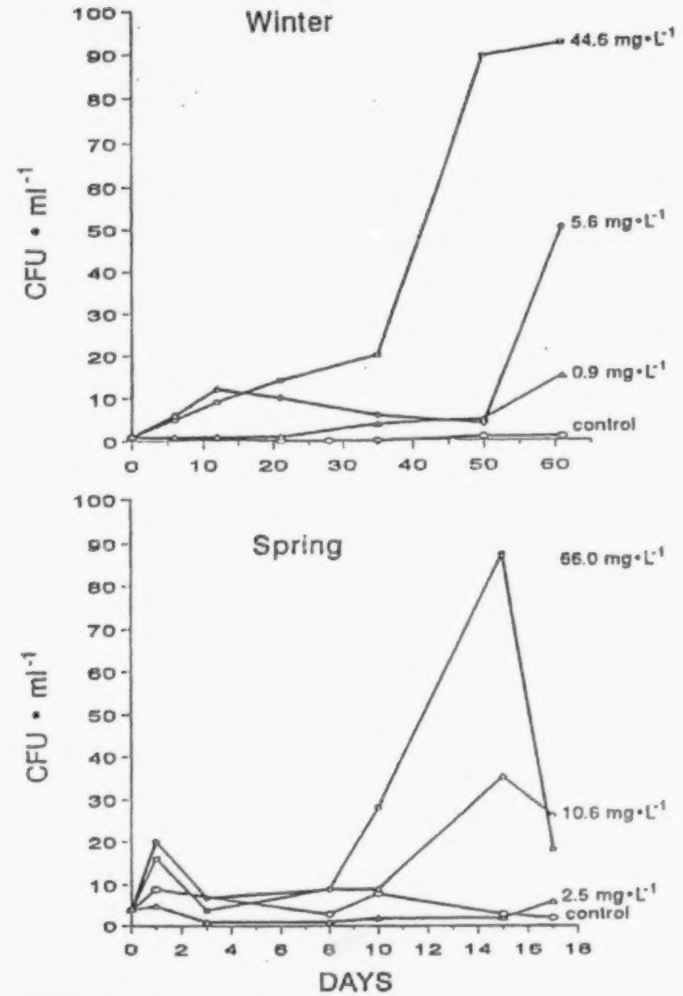

Fig.5 Growth of oil-degrading bacteria in tanks contaminated with various oil concentrations (maximum concentrations are indicated).

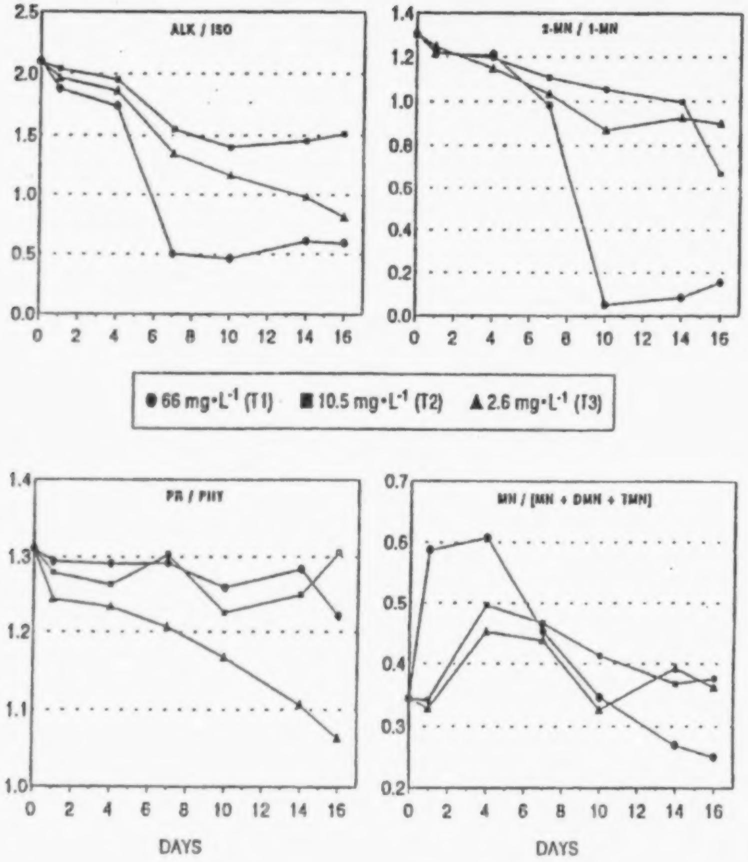

Fig.6 Spring experiment: oil biodegradation in tanks contaminated with various concentrations of dispersed oil (See *Material & Methods* for hydrocarbon ratios).

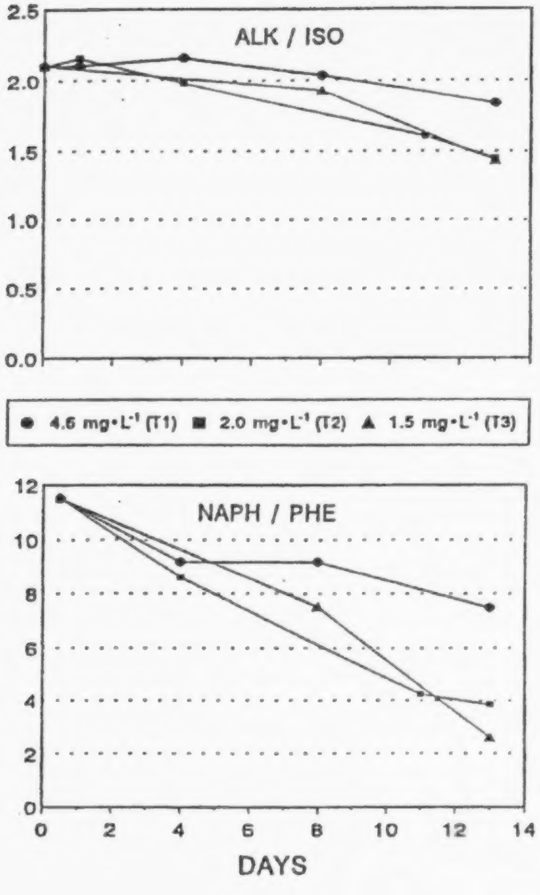

Fig.7 Winter (short-term) experiment: oil biodegradation in tanks contaminated with various concentrations of dispersed oil (See *Material & Methods* for hydrocarbon ratios).

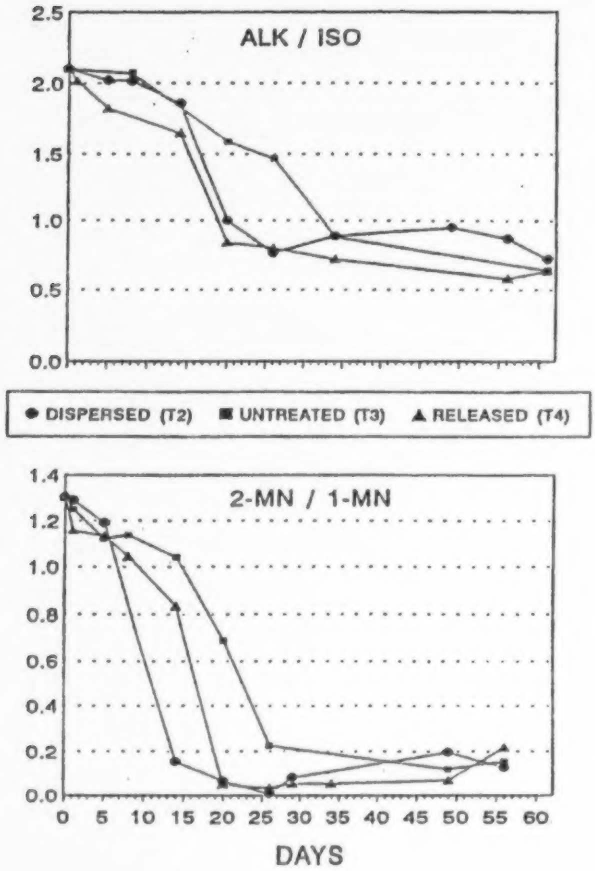

Fig.8 Winter (long-term) experiment: oil biodegradation with various oil treatments. (See *Material & Methods* for hydrocarbon ratios).

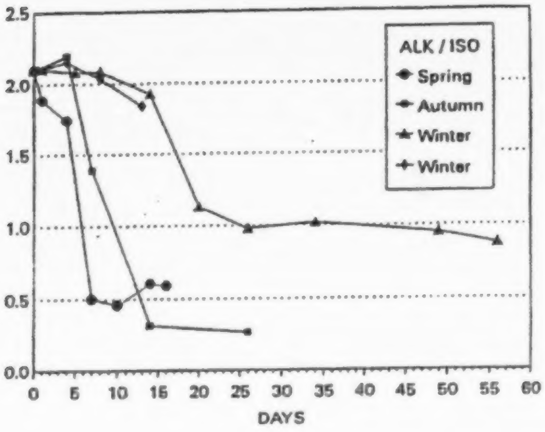

Fig.9 Oil biodegradation over the four mesocosm experiments. Data originate from the most heavily oiled tanks (dispersed oil) of each experiment.

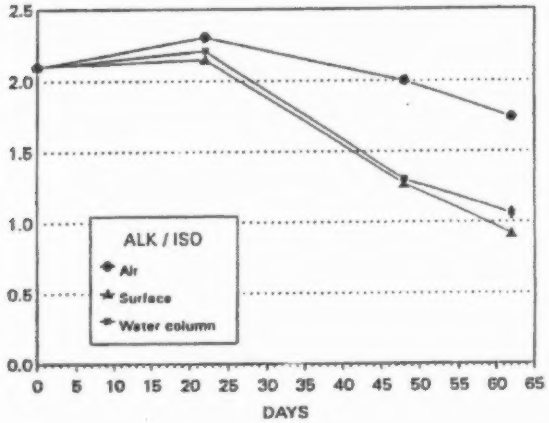

Fig.11 Winter (long-term) experiment: biodegradation of crude oil at different immersion levels (oil adsorbed on cotton strips).

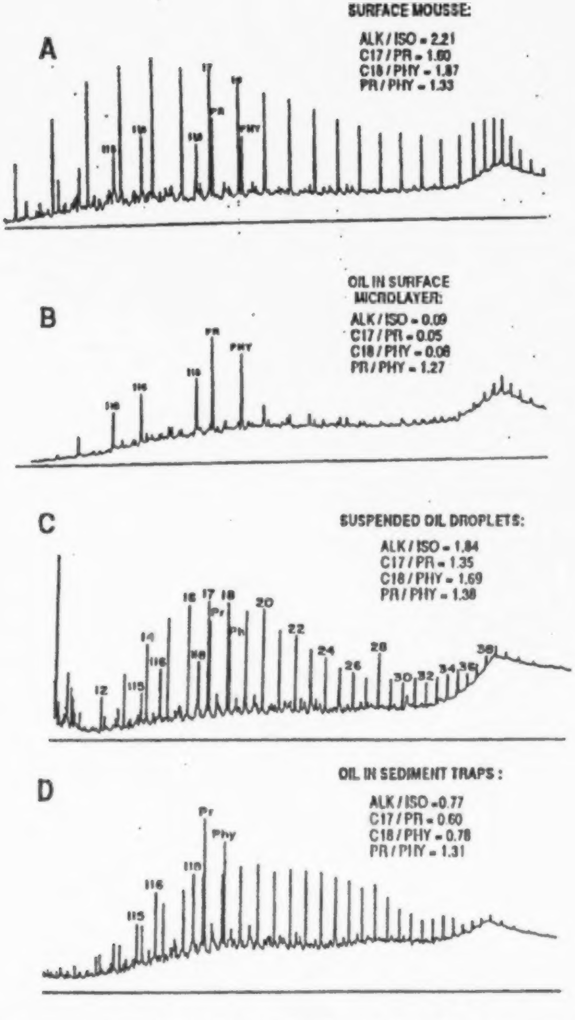

Fig.10 Gas chromatograms of the aliphatic fraction of oil residues recovered at the end of the autumn experiment (A;B) and the short-term winter experiment (C;D). Hydrocarbons are identified by the number of carbons: n−heptadecane (17); n-octadecane (18); isoprenoids: i15, i16, i18, pristane (PR) and phytane (PHY). (See *Material & Methods* for hydrocarbon ratios).

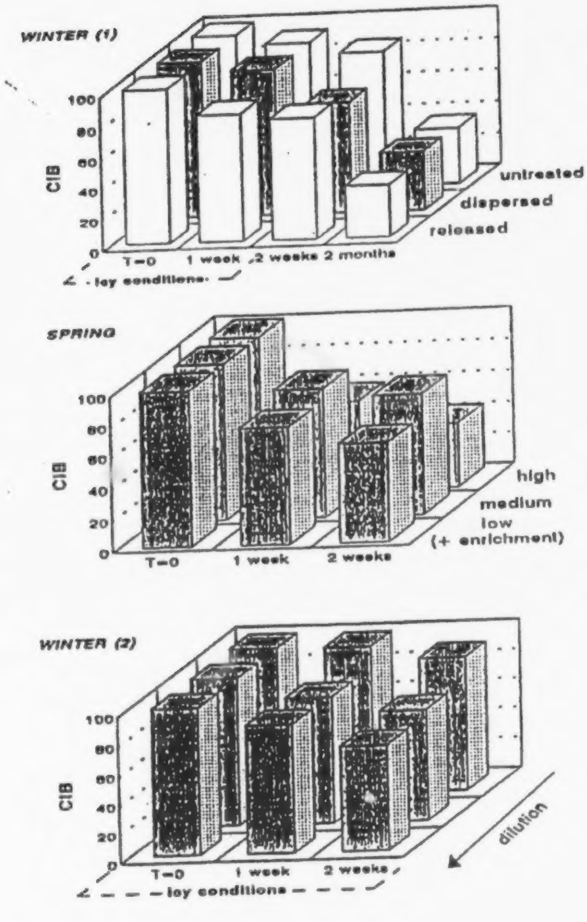

Fig.12 Combined Index of Biodegradation (CIB) calculated from both dispersed oil (gray surfaces) and untreated/released crude oil (plain surfaces). The precision on CIB calculation is estimated to ± 15. (CIB formula in *Material and Methods*).

THE IML BENTHOCOSM: A MESOCOSM FACILITY FOR THE STUDY OF DEEP SOFT BOTTOM BENTHIC ENVIRONMENTS

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Bulk concentration measurements are probably inadequate for evaluating the toxicity of a chemical substance in dynamic living marine sediments. Diagenetic zonation over small depth intervals may lead to alteration, mobility and redistribution, aided by benthic fauna activity (biodiffusion), and development of lateral and vertical heterogeneity (Silverberg & Sundby 1990). For basins such as the 350m deep Laurentian Trough, where particulates naturally accumulate, ship-based spot sampling cannot truly delineate the small-scale, non-steady state mechanisms governing the chemistry of natural substances and contaminants in sediments. Continuous access to living sediment in the laboratory is preferable. The 35 atmosphere depressurization involved in raising sediment from the floor of the Trough was thought, however, to be too great for survival of most benthic organisms.

The discovery that organisms in boxes of mud recovered from over 200m depth in the Oslofjord could survive well over 6 months in mesocosms at the Solbergstrand Marine Research Station (Berge et al. 1986) encouraged our own benthic mesocosm ("benthocosm") project, to see if a reasonable facsimile of the Laurentian Trough benthos could also be maintained in the laboratory for close scrutiny and controlled experimentation. Since 1990, four series of triplicate benthocosms have been maintained for periods of 5-14 months.

The benthocosm facility at the Institut Maurice-Lamontagne (IML) represents a compromise between the large open-circulation Solbergstrand facility and the 3-box, closed-circulation system ("boxcosm") developed at the Netherlands Institute for Sea Research for simple pollution experiments (Duineveld et al. 1991). Rather than serving as a water bath to house separate boxcores, the 1 X 1 X 0.7 m basins at IML are filled using five 45 X 45 cm cores (the sediment in the fifth box is subdivided in 10 cm slices to fill the gap along two sides of the first four cores). The double-walled basins and covers are of fiberglass construction with a Gelcoat interior surface. A ½ H.P. chiller pump maintains a constant flow of 4.5°C water through the wall jackets of all three basins, providing cooling across all four walls and base of the basins. An independent 600 L volume of overlying seawater is used for each benthocosm basin. The circuit consists of a ¼ H.P. chiller pump, in-line UV lamp and cartridge filter (5 µm), an insulated plastic reservoir receiving most of the circulation, and a bleed off diffuser-drain circuit to exchange overlying water at approx. 2000 L/day. A 0.5 mm drain screen limits the flushing of small macrofauna or larvae out of the basin. Before use the basins are washed using biodegradable soap, rinsed, and flushed with seawater for several days. The overlying water is analyzed routinely for nutrient salts, particularly ammonia. Reserve bottom water, although filtered at sea, sometimes developed significant bacterial growth. We have experimented with 18m depth filtered seawater from the continuous source at IML, made up to the 34.5 ppt salinity of bottom water using Instant Ocean™ with no evident ill effects. To limit the buildup of unmeasured substances, one-third of the overlying water is replaced every 2-3 weeks.

To minimize thermal shock, sediment collection is carried out in the fall or spring, when temperature stratification is not well developed. All material to date has been recovered from 350m depth in the center of the Laurentian Trough off Rimouski. A 0.25 m^2 boxcover, with an internal 45 X 45 cm metal liner is used to collect blocks of sediment 35-45 cm thick. Once the corer is gently landed on deck, a plastic plate is inserted at the base of the core. The box is disjoined and the corer frame hauled up and set aside. The box is then lifted away from the internal liner. Bottom water is gently added to the overlying water to fill the internal liner, an inflated plastic bag is added to take up air space and a top plastic plate is used to cover the sample. Packing ribbon is then used to secure the two plastic plates in place and the sealed liner box is hoisted, washed of external mud, and lowered into the 4°C transfer container. The entire procedure, after some practice, leaves the sediment exposed to ambient air temperature for less than 10 minutes. Each day, five boxes are collected and stored in the same manner before the ship returns to port, whereupon the transfer container is shipped immediately by truck to the laboratory. Some vibrational disturbance is inevitable.

At the Institute the sealed boxes are hoisted out of the transfer container and gently lowered into the benthocoms. The boxes are then slid up from the sediment blocks and removed entirely. The basin is covered and left until the water clears (48 - 72 hrs). The water level is then brought up to 20-30 cm and opened to circulation. Feeding is carried out by injecting a 50 ml suspension of homogenized plankton of known carbon content using a syringe to evenly disperse the suspension close to the sediment surface while the circulation is temporarily halted.

Monitoring procedures included visual and photographic observation, time-lapse video, whole-sediment oxygen demand, nutrient salt concentrations in overlying water, macrofauna and meiofauna abundances, and bacterial numbers and activity. The results of our experiments demonstrate that day-to-day close observation of organisms, sediments and the biogeochemistry of coastal sediment from several hundred meters depth can feasibly be carried out in the laboratory. The 1 m^2, closed-circulation, water jacket-insulated basins at the IML facility has been shown to preserve quite well the principal macrofaunal abundances and species composition of the deep Laurentian Trough benthos for periods exceeding a year. Meiofaunal abundances are likewise maintained, as are bacterial numbers, heterotrophic and exoenzymatic activities. Oxygen uptake rates also remain within the range observed for freshly-obtained sediment cores, while oxygen penetration depths in the sediment stabilize at about twice the thickness of the in situ aerobic layer. Buildup of nutrient salts in the overlying water can be controlled by bi-monthly replacement of a third of the bottom water.

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Although application rates vary depending on which insects need to be controlled, typical rates range from 0.5 to 2.0 oz a.i./acre. If such rates were applied directly to the surface of a 0.5 to 1.0 m deep pond (or a littoral enclosure) they would translate into water concentrations of 3.5-7.0 µg/L and 14-28 µg/L for 0.5 and 2.0 oz a.i./acre, respectively.

Diflubenzuron interferes with the molting process in susceptible, chitin-producing organisms. The exact site and mode of action is still poorly understood, although it is generally accepted that diflubenzuron interferes with chitin synthetase, the final enzyme in the pathway by which chitin is synthesized from glucose. Larval and immature stages of chitin-producing organisms are most susceptible to diflubenzuron due to the frequency of molting cycles. Exposed larvae are unable to cast their exuviae, resulting in death from either starvation (malformed larvae ar unable to feed), or rupture of the new, delicate, malformed cuticle (Eisler, 1992).

The objective of this study were primarily three-fold: (1) to describe the chemical persistence and distribution of diflubenzuron in a littoral ecosystem, (2) to determine the toxicity (direct effects) of diflubenzuron to aquatic invertebrates, and (3) to assess the indirect effects of diflubenzuron on larval fish growth, presumbly resulting from reductions in invertebrate prey populations. The study was a cooperative research effort between scientists from the University of Wisconsin-Superior and the U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota. This paper will focus on the second objective and will discuss the effects of diflubenzuron on aquatic invertebrate populations in littoral enclosures by describing treatment effects on zooplankton, benthic macroinvertebrate, and emerging insect taxa.

MATERIALS AND METHODS

Study site

The study was conducted in a set of littoral enclosures which had been previously installed in a 2-ha pond in northern Minnesota (see Brazner et al., 1988 for details). Each enclosure was 5 m wide and extended 10 m from the shore into the pond. The average depth at the deep end was ≈ 1.3 m. The aquatic plant community in the littoral zone was dominated by *Thypa latifolia*, *Equisetum sp.*, *Chara* sp., and *Potamogeton spp*.

Pesticide Application

Four diflubenzuron concentrations were evaluated in a randomized block design. This design consisted of two untreated controls and one replicate of each pesticide concentration in each of two blocks of six experimental units. The four treatment levels were chosen to meet the following objectives:

0.7 µg/L: a new effect concentration [a potential 5% spray drift concentration];
2.5 µg/L: a low effect concentration [a high spray drift concentration];
7.0 µg/L: an intermediate effect concentration [direct overspray at low application ratel]; and
30.0 µg/L: a catastrophic effect concentration [direct overspray at high application rate].

The pesticide was applied as a uniform surface spray using Dimilin® WP-

25, a wettable powder formulation of diflubenzuron. Two pesticide applications were incorporated over the course of the study season. The first application took place on July 9, and the second on August 11, 1992.

Sampling

Benthic macroinvertebrates: Benthic macroinvertebrates were sampled on 9 dates beginning on June 25 (day -13) and ending on September 4 (day 57), 1992. Samples were collected using a modified water-column substrate sample to a depth of approximately 10 cm, as well as integrated water column sample. Submerged macrophytes within the sampler's diameter were also collected. Four samples were collected from each enclosure, pooled, sieved through a 595 µm mesh screen, rinsed into 1-L glass jars, and preserved with 10% formalin solution. Samples were later sorted to remove macroinvertebrates from the debris. Organisms were identified to the lowest practical level.

Insect emergence: Emerging insects were collected on a total of 16 dates between June 29 (day -10) and September 8 (day 61), 1992 using floating, pyramid-shaped emergence traps. Each trap measured 50 x 50 cm at the water surface. An adhesive-coated (Tangle-Trap™) plexiglass collection plate, measuring 18 x 18 cm, was positioned over the top opening of the trap. Two traps were set in each enclosure and the adhesive-coated sample plates were left undisturbed for ≈ 96 h after which time they were collected and replaced with new plates. Insects were later removed from the adhesive-coated plates using turpentine and preserved with 70% ethanol until identified and enumerated.

Zooplankton: Zooplankton were sampled on 10 dates between June 24 (day -14) and September 15 (day 68), 1992. Samples were collected using inverted funnel traps, each equipped with three 15-cm diameter glass sampling funnels (Brazner et al., 1988). Four traps were placed in each enclosure on each sample day. Samplers remained in the enclosures for a 24-h period. Samples from the same enclosure were pooled and preserved with and 8% sucrose-buffered formalin solution. Samples were later decanted through a 35-µm mesh screen and diluted with water to 25, 50 or 100 ml for enumeration and identification. Five 1-ml subsamples from each sample (enclosure) were counted.

RESULTS

Benthic Macroinvertebrates

The pre-application community was dominated by Chironomidae, Mollusca, and Annelida, which together represented more than 95% of the total macroinvertebrate abundance. Other taxa present in the littoral community included Ephemeroptera, Odonate, Trichoptera, Coleoptera, other Diptera, Amphipoda, Nematoda, and Hydrachnida.

Only Insecta displayed a significant adverse response to the diflubenzuron treatment. Of the various insect taxa which displayed sensitivity to diflubenzuron, only Chironimidae and Ephemeroptera were present in high enough abundances to allow for detection of statistically significant changes. The abundance of larval Chironomidae was significantly reduced at 30.0 µg/L on days 15 and 28 post-application. Corresponding reductions in abundance relative to controls were 79 and 69%, respectively. There was also a noticeable reduction in abundance at the 7.0 µg/L treatment on days 15 and 22, corresponding to decreases relative to controls of 58 and 67%; only the day 15 reduction was statistically significant. Recovery was observed at the 7.0 µg/L treatment by day 28. No adverse affects were noted at the 0.7 and 2.5 µg/L treatments. Significant reductions in populations of Ephemeroptera were also observed at the 7.0 and 30.0 µg/L diflubenzuron treatments. Post-application reductions in abundance, relative to controls, ranged from 69 to 100%. Effects observed on most insect taxa after the second diflubenzuron application were similar to those observed after the first application.

Insect Emergence

Total insect emergences was significantly affected at the 2.5, 7.0 and 30.0 µg/L treatments within 4 to 8 days of the first diflubenzuron application. Maximum impacts were observed on day 8 post-application for the 2.5 and 7.0 µg/L treatments (87 and 96% reductions relative to controls, respectively) and on day 16 post-application for the 30.0 µg/L treatment (99% reduction relative to controls). None of the impacted populations had completely recovered by the time of the second diflubenzuron application on day 33. No adverse effect was noted at the 0.7 µg/L treatment. The response in total insect emergences after the second diflubenzuron application was similar to that observed after the first application.

The majority (96%) of emerging insects captured during this study belonged to the family Chironomidae. As a result, very few differences were noted between the response of this taxon and the response of the combined group, all Insecta. Maximum reductions, relative to controls, observed at the 2.5 µg/L treatment, were 89 and 90% eight days after the first and second diflubenzuron application, respectively. In both cases, the population recovered to near control levels within 8-12 days. Few effects were observed on other insect taxa due to the low numbers of such organisms caught in the emergence traps over the course of the experiment.

Zooplankton

The Cladocera proved to be sensitive to diflubenzuron even at the lowest treatment concentration. The mean abundance of Cladocera in 0.7 µg/L enclosures was reduced by 89% six days after the first application. Populations remained lower than control levels until day 29. Cladocera numbers again dropped significantly below control levels after the second application and did not recover by the end of the study. The remaining three treatment concentrations (2.5, 7.0, and 30.0 µg/L) all had substantial and prolonged effects on the cladoceran community. Mean population abundances in all three treatments were reduced by 92 to 99% by day 6 post-application. Abundances remained low and displayed no sign of recovery during the remainder of the 56-day long experiment.

The response of the Copepoda community to the diflubenzuron treatments was very similar to that observed for the Cladocera. At 0.7 µg/L, total copepod abundance was significantly reduced (to 70% of controls) on day 6 post-application. However, the total copepod abundance recovered to near control levels by day 12, the next sampling date. This response was primarily due to a rapid recovery of juvenile copepod stages (copepodites and nauplii), which were reduced by 83% on day 6 post-application. There was no apparent adverse effect after the second 0.7 µg/L application. At 2.5 µg/L, total Copepoda abundance was reduced by 93% relative to control levels, but recovered to within 68% by day 29. Impact after the second application with 2.5 µg/L resulted in an 88% reduction on day 40; population abundances recovered to 46% of controls by day 56. At the 7.0 and 30.0 µg/L treatments, copepod numbers were reduced by 82 to > 99% from day 6 onwards.

Juvenile copepods made up the majority of the total Copepoda community. Trends were therefore very similar to those observed for the Copepoda community as a whole, but maximum impacts generally were greater and recoveries faster. Population abundances were reduced to only 1% of control levels on day 6 at all concentrations ≥ 2.5 µg/L. Juvenile copepod abundance at 2.5 and 7.0 µg/L recovered to 70 and 77% of control levels on day 29, respectively. Abundances at the 30.0 µg/L treatment remained at < 1-5% of controls from day 6 post-application to the end of the study.

Rotifer populations displayed no signs of direct toxicity to diflubenzuron.

DISCUSSION

Crustacean zooplankton were the most sensitive taxa sampled. Cladoceran abundance was adversely affected at even the lowest test concentrations, 0.7 µg/L diflubenzuron. The maximum reduction in abundance was 89%. Copepoda were similarly affected. The lowest observable effect concentrations for Copepoda (LOEC) was again 0.7 µg/L, with maximum reductions in adult and juvenile populations of 70 and 83%, respectively. Recovery of both Cladocera and Copepoda at the 0.7 µg/L treatment was observed within 2-4 weeks (Table 1). The no observable effect concentrations (NOEC) could not be determined. Rotifers appeared to be insensitive to diflubenzuron within the range of concentrations tested.

Table 1. Summary of no observable and lowest observable effect concentrations for the impact of diflubenzuron on aquatic invertebrates in littoral enclosures.

TAXA	NOEC(1) (µg/L)	LOEC(2) (µg/L)	Maximum reduction at LOEC (%)	TIME TO RECOVERY AT LOEC(3) (days)
Cladocera	<0.7	0.7	89	12-29
Copepoda				
- adults	<0.7	0.7	70	22-29
- juveniles	<0.7	0.7	83	12-29
Rotifers	30.0	>30.0	--	--
Chironomidae				
-larvae	2.5	7.0	67	28
-adults	0.7	2.5	89	16

(1) NOEC: no observable effect concentration (nominal exposure).

(2) LOEC: lowest observable effect concentration (nominal exposure)

(3) After the first diflulbenzuron application.

The only benthic macroinvertebrate group which appeared to have been adversely affected by the diflubenzuron treatments was the Insecta. Effects were most noticeable on the most abundant taxa; the Chironomidae. The NOEC and LOEC for this taxon were 2.5 and 7.0 µg/L diflubenzuron, respectively. Effects at the 7.0 µg/L treatment were transient in nature; impacted populations generally returned to control levels within 3-4 weeks. Effects observed at the 30.0 µg/L treatment were substantially more pronounced and persistent.

Emergence was a more sensitive test endpoint for effects on Chironomidae than was larval abundance. This was not surprising, considering the mode of action of diflubenzuron. The LOEC for Chironomidae emergence inhibition was 2.5 µg/L. At this treatment concentration, emergence was reduced by up to 89%, relative to controls, with recovery observed within 16 days. Recovery was not observed at the 7.0 and 30.0 µg/L treatments. The NOEC was 0.7 µg/L (Table 1).

Since the 0.7 µg/L treatment approximated an average environmental concentration in a littoral ecosystem which could result from an aerial spray drift of 5-10%, it can be assumed that, under "normal" operational conditions, the use of diflubenzuron near aquatic habitats poses a small, but significant, risk to natural crustacean zooplankton population. Aqueous concentrations ≥ 2.5 µg/l, which could result from either very high spray drift levels or from direct overspray, would pose a significant risk to most crustacean and insect taxa inhabiting littoral ecosystems.

CONCLUSIONS

- Cladocera and Copepoda were the most sensitive invertebrate taxa. Both were significantly impacted at 0.7 µg/L diflubenzuron, the lowest test concentration.
- At 0.7 µg/L, all impacted invertebrate populations recovered within 2-4 weeks post-application.
- At 2.5 µg/L, Cladocera did not recover by day 33 (time of second application), or by day 56 (last day of study). Chironomidae emergence was inhibited between days 4 adn 16.
- At 7.0 and 30.0 µg/L, there were significant reductions in both larval and adult Chironomidae. Most crustacean zooplankton and insect taxa were substantially reduced for most of the experimental period. There were no effects on rotifers, molluscs, or annelids.
- Normal use of diflubenzuron near aquatic habitats poses a small, but significant, risk to sensitive crustacean and insect species.

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MODELLING POPULATION EXPOSURE-RESPONSE FUNCTIONS FOR USE IN ENVIRONMENTAL RISK ASSESSMENT.

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Effective environmental management requires detailed knowledge of the probable individual, population and ecosystem responses to identified environmental hazards. While much is known about the short-term toxicokinetics and physiologial impacts of many toxic chemicals, little is known about the long-term responses of exposed populations. This occurs, in part, because of the costs and difficulties associated with completing long-term studies. Laboratory assay data provide an expedient means of determining the toxicity of chemicals to selected organisms and, through the judicious selection of both organisms and chemicals, their relative toxicity. Ideally, these results could be exptrapolated to predict the effects of toxicant exposure on natural populations. Unfortunately, laboratory studies are not designed for extrapolation into the complex, dynamic environment occupied by natural populations. This has lead some (Cairns, 1980; Hendrix, 1982) to question the justification for using laboratory results to predict toxicant effects on natural systems.

In the absence of long-term studies and in the face of questions concerning the adequacy of laboratory based projections, it is argued that modelling provides a credible means of predicting the long-term responses of exposed populations. An individuals based Atlantic salmon (*Salmo salar*) population dynamics model of the riverine life-stage (Power and Power, 1993) was used to measure the recovery time in a population subjected to concentrations and durations of copper exposure characteristic of an accidental release of mine tailings. The model separates the density-dependent mortality phenomena that regulate parr numbers from the random environmental influences that regulate population growth and size. The original model was altered to include laboratory-derived age-specific acute-toxicity data. The date were drawn from Dixon (1980) and consisted of the mortality data for rainbow trout (*Onchorynchus mykiss*) exposed to varying concentrations of copper [Cu]. Close correlations between genera and species toxicity responses (Suter et al., 1983) and the reported similarity in threshold LC50s, 48 and 47 μg/L respectively for like sized juvenile salmon and rainbow trout, argue the data are a suitable proxy.

A basecase version of the model was run for comparative purposes. The results of the basecase depend only on the natural variability modelled for the environment and do not include any consideration of the pulse exposure to Cu. The experiments consisted of hypothesized short-medium and long-duration pulse exposures to Cu. Plots of the time to recovery curves estimated from the 12-, 24- and 48-hour response experimental date, as a function of concentration, are given in Fig. 1. Response curves were estimated using the experimental data. Statistical criteria suggested the selection of the three parameter Gompertz model as the best description of the time

to recovery: $RT = \alpha \exp[-\exp(\beta - \gamma K)]$ where RT defines response time in years, K the Cu concentration in μg/L and α, β, γ are estimated parameters.

The model results can also be presented in terms of population-level exposure-response curves by comparing the basecase population figures with those of each of the experimental runs to determine the cumulative mortality at a given concentration. As with other toxicity data, threshold LC50s can be defined for the population-level responses. The population-level threshold LC50, 160 μg/L, was significantly less than the laboratory derived value of 225 μg/L. The results underscore the dangers of extrapolating laboratory data to natural populations as a means of assessing the in-situ mortality resulting from pulse exposures. This suggests that population and age-size structure information be used in conjunction with toxicity test data to construct a weighted average LC50 that would be more reflective of likely population mortality than a laboratory-derived LC50.

The study concludes that laboratory toxicity data were not suited to predicting the population-level consequences or risks associated with pulse exposures to toxicants. Such data are static and too controlled with respect to biotic and abiotic factors to make them relevant for extrapolation to the environment. Modelling, however, allowed the estimation of population-exposure response functions defining the time to recovery as a function of concentration over a range of concentrations and time exposures and could be used as a means of assessing population level recovery times and risks. The estimated time to recovery for parr and smolt sub-groupings were, respectively, 15 and 21 to 22 years. This is in excess of the single life-cycle time that might intuitively be predicted. Reproductive lag effects extend the recovery time and increase the observed post-disturbance variability in observed population levels. Accordingly, post-disturbance monitoring programs for the detection of changes in population levels will be more difficult insofar as larger samples will be required to obtain the appropriate degree of statistical confidence.

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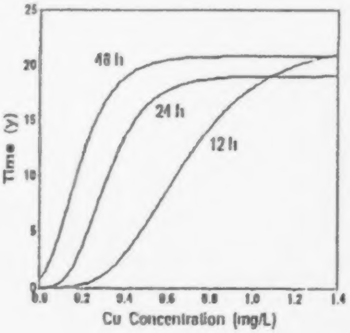

Fig. 1. Plots of the time to recovery for smolt abundance, as a function of concentration, estimated in the 12-, 24-, and 48-hour experiments.

ALGAE AS TOOLS IN AQUATIC TOXICITY TESTS

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Laboratory bioassays with cultures of algae have been used since the 1950's to test potential effects on algal growth of nutrients, various contaminants, and complex effluents. Photosynthetic rate has also been used as the criterion of algal response, but to a lesser extent than growth rate. Recently, I have explored the use of light saturated photosynthetic rates to assess the potential effects of dredged sediment disposal on primary production in the receiving waters. Algal cultures or natural phytoplankton samples were incubated with 14-C bicarbonate in the presence of sediment, and 14-C labelled photosynthate was extracted with dimethyl sulfoxide at the end of the exposure. In comparison with sediment elutriates, direct sediment bioassays showed Lake Ontario sediments to be significantly more toxic to primary producers.

In a comparison of growth and photosynthesis EC50 values for copper and vanadium toxicity, EC50 values from growth experiments were lower than from photosynthesis experiments but became more similar with increased duration of the photosynthesis experiments. For a given species, there was a fixed relationship between the two EC50's, hence photosynthesis EC50 values may be used to predict growth EC50's.

The choice of algal species for the bioassay is important because large differences have been documented in responses to various contaminants among genera, species, and ecotypes. Different ecotypes, or physiological races, exist in about 50 species of algae examined to date, and differ in traits such as maximal growth rate, vitamin requirements, and tolerance of acid pH, metals, and organic contaminants. Given the fast growth rates characteristic of algae, and the substantial differences in growth rates reported among the physiological races, the opportunity exists for fast evolutionary responses of phytoplankton communities to stress. I have documented differences in sensitivity to various contaminants at the genus, species, and strain (ecotype) level. For example, in the sediment bioassays, responses differed among species of algae, as well as between cultures and natural phytoplankton populations. In 12 species of algae and cyanobacteria, large differences were apparent in vanadium toxicity photosynthesis, and the responses were not similar among members of a given taxonomic group. In three strains of *Scenedesmus acutus f. alternans*, growth EC50's for copper were about 4 times higher in B-4, the most tolerant strain, than in the copper- intolerant strain, UTEX-72. Photosynthesis EC50's for the same two strains different by a factor of 3.2

The physiological state of bioassay cultures must be standardised in order to avoid the confounding effects of nutrient status, (e.g. differences in cell P, N or C metabolism) on algal responses to toxic substances. The P state of cultures was the most important factor controlling the extent of vanadium toxicity to growth and photosynthesis in several species of diatoms, green algae, and cyanobacteria. Apparently orthovanadate, the predominant form of vanadium in our experiments, competed with orthophosphate for uptake. Photosynthesis of P-sufficient cells was not inhibited by vanadium but a 50% inhibition was apparent in P-deficient cells that had about 10% of the maximal cellular P-quota. In experiments on the effect of P-state to copper toxicity, photosynthesis in P-loaded cells of UTEX-72, the copper-intolerant strain of *Scenedesmus*, was significantly less depressed by copper than in P-starved cells but P-state had no effect on copper toxicity in the two copper-tolerant strains, B-4 and X-Cu. In contrast, N-starvation affected copper toxicity to photosynthesis in al three strains: B-4 was less tolerant but X-Cu and UTEX-72 were more tolerant when cells were N-deficient than when N-sufficient. The B-4 strain relies on internal Cu detoxification but X-Cu excludes copper. In species that rely on metal exclusion as the tolerance mechanism, N starvation may enhance extracellular fibril production, and hence increase metal binding and tolerance.

Given the existence of species and strain-specific sensitivity to contaminants, it is likely that tolerant taxa of phytoplankton replace sensitive species or strains in lakes subjected to chronic pollution. Since the physiological condition of the species is also an important factor, the use of natural phytoplankton communities in bioassays may be preferable to single species cultures. The ability of phytoplankton to adapt to stres was apparent in experiments on the effect of pH shock on photosynthesis in two lakes: Plastic Lake, which is undergoing acidification and St. Nora, which is not impacted. Lakewater samples were collected at different times of the year and the pH was adjusted in the laboratory to six values in the 6.5 to 4.0 range. Depression of photosynthesis by pH below ambient was more severe in Lake St.Nora regardless of season. In contrast, depression was less severe in Plastic Lake, particularly in spring phytoplankton communities. We concluded tentatively that phytoplankton in Plastic Lake was adapted to acis shock. To some extent this conclusion is augmented by the discovery of three acid-tolerant species of phytoplankton *(Chlamydomonas vernalis, Nitzschia sp. and Oscillatoria utermoehlii)* which we isolated into culture from Plastic Lake.

A simple microcosm bioassay is described in which lakewater was subjected to the toxic substance, copper in this case, at several concentrations, and both phytoplankton biomass and species numbers scored at the end of as 10- 14 day growth period. In Clearwater Laker, and acidified and metal contamined lake near Sudbury the decrease in biomass and species diversity was significantly less severe over the selected range of copper concentrations than in Plastic Lake which is not contaminated with copper.

In summary, for an order of magnitude assessment of potential effects of a toxic substance on ecosystem function (algal biomass, primary productivity), the standard *Selenastrum capricornutum* bioassay is adequate. For a convenient and fast assessment, EC50 values based on light-saturated photosynthetic rates can be used to predict growth EC50's, but for some toxic substances at least, the results will vary depending on the choice of species of algae raises questions on the validity of using cultures of a single species to assess potential effects of toxic substances on ecosystem function. A more realistic assessment of potential impact on ecosystem function and structure may be obtained from laboratory micro- or mesocosms in which changes in algal biomass and species diversity are determined after exposure for about two weeks to a range of concentrations of the toxic substance.

BOILER BLOWDOWN TOXICITY REDUCTION TESTS

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Boiler blowdown effluent from both nuclear and thermal stations was often toxic to both rainbow trout and *Daphnia magna* in toxicity tests conducted during the monitoring phase of the Ontario Municipal Industrial Strategy for Abatement (MISA) Program. The toxicity of boiler blowdown effluent may have been caused either by its low ionic content or through interactions of the low ionic content and elevated metal concentrations. Accordingly, follow-up studies were conducted to evaluate mechanisms of reducing the toxicity of boiler blowdown effluent. Addition of calcium ions, either by adding calcium chloride ($CaCl_2$) or recirculation over a limestone filter for ≈ 12 h, largely eliminated the toxicity of boiler blowdown effluent to trout, but the treated effluent was still toxic to *D. magna*. Addition of humic acid (≈ 20 mg/L as dissolved organic carbon) effectively mitigated the toxicity of boiler blowdown effluent to both *D. magna* and rainbow trout. The toxicity of boiler blowdown effluent thus appears to result from the interactions of its low ionic content and elevated metal concentrations, but addition of humic acid and $CaCl_2$ would effectively reduce this toxicity.

ENVIRONMENTAL FATE AND EFFECTS OF BLEACHED PULP MILL EFFLUENTS: FOLLOW-UP STUDIES ON DIOXIN, EROD INDUCTION, AND THE ROLE OF DIETARY PATHWAYS. P. Kloepper-Sams[1], L. Steeves[2] and J. Stegeman[3]. 1 The Procter & Gamble Co., Cincinnati, OH; 2 Weyerhaeuser Ltd., Grande Prairie, AB; 3 Woods Hole Oceanographic Institution, Woods Hole, MA.

As part of a multi-year (1990-92) chemical biological assessment of fish populations exposed to bleached kraft mill effluent near Grande Prairie in northern Alberta, we followed responses in the river ecosystem to changes in mill process (e.g., shift from molecular chlorine to chlorine dioxide). Although no population-level impacts were observed, several endpoints were analyzed further: dioxin/furan body burdens in the mountain whitefish *Prosopium williamsoni*; hepatic cytochrome P4501A ("MFO") in this species; and the role diet may play in uptake of P4501A inducer(s). Dioxin levels have declined steadily since replacement of molecular chlorine; data from 1993 will be reported. P4501A protein and associated enzyme activity (EROD) in whitefish collected near the mill discharge declined significantly from spring 1991 to spring 1992, with further declines by spring 1993. Impacts of a scheduled maintenance shutdown on EROD values will be discussed in light of hypotheses on fish uptake and depuration of inducers. To examine the route of uptake of inducing compounds, P4501A in intestinal samples was visualized by enhanced chemiluminescence, and P4501A cellular localization in gill, intestinal, and liver tissues was determined by immunohistochemistry. These results will be compared with dioxin body burdens. Potential implications for environmental monitoring and regulation of pulping effluents will be discussed.

APPORTS RÉPÉTÉS DE LISIERS ET PROPRIÉTÉS CHIMIQUES D'UN SOL LOAM ARGILEUX NEUBOIS

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RÉSUMÉ

Au cours d'une expérience de longue durée (1978-1992), une étude a été effectuée sur les effets de l'application répétée du lisier de porc sur l'extractibilité du C organique, de l'N total, du P total et du P disponible. Le lisier (0, 30, 60, 90 et 120 mg ha^{-1}) était incorporé par aspersion en bandes à un loam argileux de la série Neubois (gleysol humique). Le maïs à ensilage (*Zea mays L.*) a été cultivé en semis direct et sans apport minéral. Des échantillons de sol ont été prélevés en 1992 par couche de 20 cm jusqu'à une profondeur de 1m. Les traitements ont affecté, tant en surface qu'en profondeur, les concentrations en C organique du sol. Des effets analogues ont été notés pour N et P totaux ($P<0.01$) jusqu'à une profondeur de 1m. Des effets très significatifs ($P<0.01$) ont été observés pour la concentration en P extractible (Mehlich-3P), jusqu'à 60 cm de profondeur. L'augmentation du contenu du sol en ces trois éléments a été particulièrement marquée pour les parcelles traitées avec les plus fortes doses de lisier de porc (90 et 120 M gha^{-1}). L'application de fortes doses de lisier de porc a donc augmenté la fertilité et amélioré la qualité du sol grâce à l'accroissement des teneurs en éléments extractibles. Cependant, l'accumulation à la surface du sol de certains éléments nutritifs potentiellement nocifs et leur migration possible dans le profil du sol peuvent affecter la qualité de la nappe d'eau souterraine et celle des eaux de surface. Il existe un lien étroit entre la perte d'azote et de phosphore dans l'environnement et la forte concentration du carbone organique à la surface du sol à partir de l'épandage de fortes doses de lisier.

Mots-clefs: Lisier de porc, C organique, N total, phosphore, migration, accumulation, contamination.

INTRODUCTION

La gestion des déchets d'origine animale a reçu, ces dernières années, une attention particulière de la part du public et des chercheurs, à cause des problèmes de pollution potentielle générés par l'application de fortes doses de fumiers ou de lisiers sans regard à la capacité de support des sols et des besoins des cultures en éléments fertilisants. La concentration de fortes densités animales sur une aire de stabulation réduite a induit une pratique courante d'épandage de grandes quantités de lisiers avec pour conséquences: l'accumulation et la migration dans le sol ainsi que la perte dans l'environnement des polluants potentiels tels que l'N, le P et le C organique comme agent participant au transport de contaminants dans le sol.

En effet, peu de travaux de recherche en agriculture ont porté sur l'accumulation du C organique, de l'N total et du phosphore et leur transport dans les couches profondes du sol suite à l'application répétée de lisier de porc, sur une longue période de monoculture sous condition de travail de sol réduit. La plupart des études menées jusqu'à présent, étaient surtout axées sur des effets de doses répétées des engrais organiques sur l'enrichissement des sols par des composés chimiques et sur le mouvement de ces composés à court terme soit 2 à

5 années (Chang et al., 1991, Sutton et al., 1978). L'emploi du lisier de porc en agriculture est une des meilleurs voies de recyclage d'importants volume d'effluents générés par l'industrie porcine au Québec et ailleurs dans le monde (Couillard et al., 1993; Gangbazo et al., 1993; Simard et al., 1993; Ndayegamiyé et Coté, 1989; Sharpley et al., 1984;). L'apport de fortes doses de fumier de bovins constitue une source appréciable d'éléments fertilisants et peut agir comme amendement organique (Sommerfeldt et Chang, 1985; Campbell et al., 1986). Cependant, une baisse de rendement du sorgho a été notée après l'emploi de fortes doses de fumier (Mathers et Stewart, 1974). Des réductions de rendement ont aussi été observées chez le maïs (*Zea mays* L.) avec l'emploi de fortes doses de déjections liquides ou solides de dindon (Sutton et al., 1986). Par ailleurs l'apport de doses élevées d'engrais organiques peut résulter en une forte accumulation de C organique, d'azote et de phosphore dans le sol et constituer de ce fait une source de pollution des sols, des eaux de surface et souterraines (Mcalla, 1974). L'objectif de la présente étude était de démontrer l'effet de 15 applications annuelles de lisier de porc sur le mouvement du C organique comme agent dynamique dans le transfert de masse du P et de l'N dans un profil de sol de la série Neubois en semis direct de maïs-ensilage ou sous conditions de travail minimal.

MATÉRIEL ET MÉTHODES

Le site expérimental est localisé à St-Lambert de Lévis, Québec, Canada et présente un sol loam argileux de la série Neubois (gleysol humique) à pH 6.2 avec un contenu moyen en carbone organique (4%), 29% Sable, 28% Argile et 43% Limon, un taux de saturation élevé en bases (% SB=84) et une bonne stabilité structurale (diamètre pondéral moyen = 3). Des doses croissantes de lisier de porc (0, 30, 60, 90 et 120 Mg ha^{-1}) ont été appliquées annuellement au printemps, selon un dispositif expérimental complètement aléatoire et cela en quatre répétitions. Les caractéristiques moyennes (1982-1992) du lisier utilisé sont présentées au Tableau 1.

Tableau 1 Caractéristiques chimiques du lisier de porc[1]

	Unités	Moyenne	Écart-type
Matière sèche	%	3.2	0.83
C organique	%	2.9	0.54
N total (%)	%	0.3	0.97
NH_4	%	0.2	0.58
P	%	0.65	0.46
K	%	1.33	0.34
Rapport C:N	%	8.3	0.38

[1] Moyenne de 12 échantillonnages (1982 à 1992).

La dimension des parcelles est de 3 m x 15 m avec une pente de 3%. Les parcelles ont reçu uniformément 4 Mg ha^{-1} en 1980, 2 Mg ha^{-1} en 1984 et 1 Mg ha^{-1} en 1991 de chaux afin de contrer une acidification générée par l'apport de lisier. Des échantillons de sol ont été prélevés avec une tarrière à tous les 20 cm jusqu'à un m de profondeur. Les échantillons ont été séchés à l'air et tamisés à de 2 mm pour fins d'analyse. Une partie des échantillons de sol a été finement moulue et tamisée à 0.5

mm pour le dosage du C organique et d'éléments totaux. Le pH et la conductivité électrique ont été déterminés dans des suspensions sol tamisé à 2 mm (rapport sol: eau distillée 1:2) agitées pendant 30 mn (Page et al. 1982). Le contenu en C organique du sol a été obtenu par digestion humide (Allison et al., 1965). Les éléments extractibles tels que le phosphore, le calcium, le potassium et le magnésium ont été mesurés à l'aide de la solution de Mehlich-3 (Tran et Simard, 1993). Le P total et l'azote total ont été extraits simultanément par la méthode de digestion à l'acide sulfurique (Rowland et Grimshaw, 1985). La capacité d'échange cationique, les bases échangeables, le taux de saturation en bases et l'indice d'adsorption du sodium ont été obtenus à l'aide d'une solution d'extraction au $BaCl_2$, 0.1M (Herdershot et Duquette, 1986). Les analyses statistiques ont été réalisées en utilisant la procédure GLM (SAS Institute, 1988). Le test de LSD (5%) a servi à différencier les valeurs des paramètres chimiques mesurées par profondeur.

RÉSULTATS ET DISCUSSIONS

Les résultats de l'analyse de variance décrivant l'impact des applications répétées de lisier de porc sur les différentes propriétés du sol sont présentés au Tableau 2.

TABLEAU 2 Analyse de la variance (valeur de F) de quelques paramètres physico-chimiques mesurés du profil de sol après une longue durée d'apport de lisier de porc.

	Profondeur (cm)				
Paramètres	0-20	20-40	40-60	60-80	80-100
C organique	507.56**	498**	20.04**	187.20**	245.26**
N total	3.34**	3632.38**	6365.31**	2313.10**	46.06**
P total	17.24**	36.62**	19.30**	35.80**	29.86**
P (Mehlich3)	3511.67**	145.42**	10.98**	5.03**	24.29**
pH	3.57*	NS	NS	NS	3.38*
SAR'	22.01*	6.91**	263.54**	11.75**	101.78**
C.E'	23097.00**	11262.00**	3093.22**	2067.08**	5838.81**

*, **, significatif au seuil de $P<0.01$ et NS=non significatif respectivement.

'Sarv: "sodium adsorption ratio"; d= $^{Na}\sqrt{}(Ca+Mg)$

'C.E: conductivité électrique

L'effet global des traitements sur le contenu en carbone organique du profil est variable selon les profondeurs. Comparativement au témoin, on constate un accroissement, soit une accumulation du carbone organique dans tout le profil de sol, de 0-20 cm à 80-100 m particulièrement avec les doses de 60, 90 et 120 Mg ha^{-1}

Figure 1.

Il existe une forte corrélation entre la concentration en carbone organique en profondeur (80-100 cm) dans le profil de sol et les doses de lisier appliquées ($0.12 + 0.0025x$; $R^2 = 0.93$). La même relation a été établie par Sommerfeldt et Chang (1985). Ainsi, lorsqu'on applique la plus forte dose de 120 Mg ha^{-1}, on obtient pour les profondeurs 0-20 et 80-100 cm des accroissements respectifs suivants pour le carbone organique: 11.8 g kg^{-1} et 2.7 g kg^{-1}. Angers et N'dayegamiye (1990) ont obtenu une valeur similaire (11.5 g kg^{-1}) dans la couche 0-15 cm après 10 années d'application bi-annuelle de 80 Mg ha^{-1} de fumier de bovins soit de 45% par rapport au témoin. La quantité d'engrais organique appliquée au sol est un facteur important pour l'accumulation de C organique dans le sol. Sommerfeldt et al., (1985) ont trouvé 45 g C kg^{-1} représentant 0.025% C par Mg ha^{-1} avec l'application de 180 Mg ha^{-1} de fumier, après 11 ans de monoculture de maïs.

L'azote total qui totalise l'azote organique et l'azote inorganique a été significativement affecté par 15 années d'application de doses croissantes de lisier de porc. L'accroissement des concentrations en azote total dans le profil de sol sont les suivantes pour les doses apportées de 0 Mg ha^{-1} à 120 Mg ha^{-1}: 620 mg kg-1; 782 mg kg^{-1}; 806 mg kg^{-1} 711 mg kg $^{-1}$; 484 mg kg $^{-1}$ respectivement avec les couches 0-20, 20-40 cm, 40-60cm, 60-80 cm et 80-100 cm (Figure 2). On observe ainsi une forte corrélation entre les doses de lisier appliquées et la concentration de N total dans la couche 80-100 cm.

Figure 2

Une telle tendance peut être source d'inquiétude pour la qualité des eaux de surface et de drainage au fil du temps car c'est à ce niveau que sont normalement localisés les drains agricoles au Québec. Chang et al. (1991) ont déterminé 2500 mg kg^{-1} N total accumulés dans la couche 30-60 cm suite à 11 années d'applications annuelles (90 Mg ha^{-1}) de fumier de bovins en culture irriguée. L'accumulation de l'azote total augmente avec la dose de lisier et la profondeur (Chang et al, 1991, Smith et al. 1980). L'effet des traitements sur le contenu en P total du sol est significatif ($P < 0.01$) dans tout le profil du sol. On observe un enrichissement en surface jusqu'à une dose de 60 Mg ha^{-1} (Fig. 3) et une accumulation à peu près linéaire avec les doses d'application pour les couches 40-60, 60-80, 80-100 cm de cet élément. L'enrichissement a varié entre 300 mg kg^{-1} dans la couche 0-20 cm à 100 mg kg^{-1} dans la couche 80-100 cm. Chang et al. (1991) ont observé qu'après 11 années d'application de 180 Mg ha^{-1} de fumier de bovins en conditions de cultures irriguées, le P total s'est accru de 1875 mg kg^{-1} dans la couche 0-60 cm.

Figure 3

Ces observations suggèrent en dépit d'une accumulation en surface qu'une migration importante de P s'est produite dans ce profil. Une fraction importante du P des lisiers est sous forme organique. Ces formes sont plus mobiles que les formes minérales (Rolston et al., 1975). Le P a pu aussi migrer par voie préférentielle par les biopores (Logan et al., 1991). La concentration en phosphore extractible (Mehlich-3) a été fortement affectée par les traitements ($P<0.01$) dans le tout le profil de sol (Tableau 2). L'accroissement a varié entre 160 mg kg^{-1} dans la couche 0-20 cm à 17 mg kg^{-1} dans la couche 60-100 cm (Fig. 4).

Figure 4

L'accroissement de teneurs en P Mehlich-3 de la couche de surface représente 97% de l'accroissement en P total. Ces résultats suggèrent qu'une grande partie du P ajouté est demeurée sous forme labile. Le P apporté au sol grâce aux applications répétées de lisiers était donc largement en excès par rapport aux besoins des récoltes. On peut donc craindre dans le cadre de la présente étude un risque d'eutrophisation des eaux de surface et la contamination de la nappe souterraine surtout à cause du mouvement ascendant de l'eau au printemps et son retrait en automne. Il y a eu donc accumulation en surface (0-20 cm) du P extractible. Simard et al. (1993) ont trouvé dans l'horizon A, 592 mg kg $^{-1}$ sous forme de P labile (55% P total) dont 112 mg kg^{-1} P soluble à l'eau soit 19% du P labile total (résine + $NaHCO_3$+NaOH) dans un sol cultivé en maïs (*Zea mays* L.). Simard et al. (1991) ont déterminé 57 mg Kg^{-1} Mehlich-3 P constituant la limite maximale de réponse du maïs. Ils ont ainsi établi une corrélation linéaire ($r = 0.76$) entre le P soluble à l'eau et le P Mehlich-3. Ceci pourrait favoriser le transfert de P sous forme particulaire par les eaux de ruissellement vers les lacs et autres plans d'eau (Sharpley et al. 1987; Bédard, 1989). De plus, le phosphore contenu dans le lisier de porc migre plus rapidement que celui trouvé dans les engrais minéraux; la matière organique présente dans le lisier favorise également ce déplacement et entraîne directement au cours du lessivage, du P dans les couches profondes du sol (Vetter et Steffens, 1981). L'engrais organique accroît la disponibilité et la migration de P dans le sol et ce mouvement de P observé dans la couche 30-60 cm serait probablement sous forme organique (Meek et al. 1982). Hannapel (1964) a suggéré que ce déplacement de P dans les couches profondes pourrait se produite sous forme de corps microbiens et de débris cellulaires. Rolston (1975) a trouvé que des esters de phosphates (P organique) de faible poids moléculaire avec un ou plusieurs groupes hydroxyles sont davantage moins fixés par le sol que le P inorganique au cours de l'infiltration de l'eau. L'optimum des rendements obtenus pour le maïs-ensilage dans le cadre de la présente étude coincide avec la dose de 60 Mg ha^{-1} (Côté 1990) et la dose de 30 Mg ha^{-1} semble être une dose environnementale correspondant à la capacité de support agronomique du sol cultivé. Nos résultats concordent avec ceux d'autres auteurs. Meek et al. 1982 trouvent que des applications supérieures à 60 Mg ha^{-1} de lisier résultent à une accumulation (30-60 cm) de P supérieur aux besoins des niveaux de phosphore.

CONCLUSION

L'application répétée de lisier de porc pendant quinze années de monoculture de maïs, a favorisé l'enrichissement en C organique, N total, P total et P extractible du profil de sol. L'importance de l'accumulation jusqu'en profondeur de ces éléments dans le sol suggère qu'une migration et un entraînement par les eaux de drainage vers les eaux souterraines soient probables. On assume aussi que la qualité des eaux de surfaces peut en être affectée. L'augmentation de la concentration en N et P totaux tant en surface qu'en profondeur suite au traitement peut être source d'inquiétude. Parmi les propriétés liées à la qualité de la solution du sol, le pH n'a pas été significativement modifié par l'apport même à fortes doses de lisier de porc car le site a été chaulé. La conductivité électrique et le rapport d'adsorption du sodium, bien que très influencés par les traitements, ne suggèrent pas à long terme une altération de la qualité de la solution du sol, ni celle des eaux de drainage. Puisque les plus forts taux d'accumulation de nutriments ont été obtenus avec les doses les plus élevées en lisier, une rationalisation de l'emploi du lisier en agriculture devrait être réalisée de façon à éviter la surfertilisation du sol et prévenir l'exportation des éléments nutritifs vers les eaux de surface et souterraines. Dans le cadre de cette étude, une dose de 30 Mg ha^{-1} de lisier serait considérée comme une dose environnementale pour le maïs car elle correspond par exemple au niveau optimal de P labile ou P extractible au Mehlich dans la série Neubois.

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REMERCIEMENTS

Les auteurs remercient le CRSNG (subvention OGP0003711) pour avoir fourni les fonds nécessaires à la réalisation de ce projet.

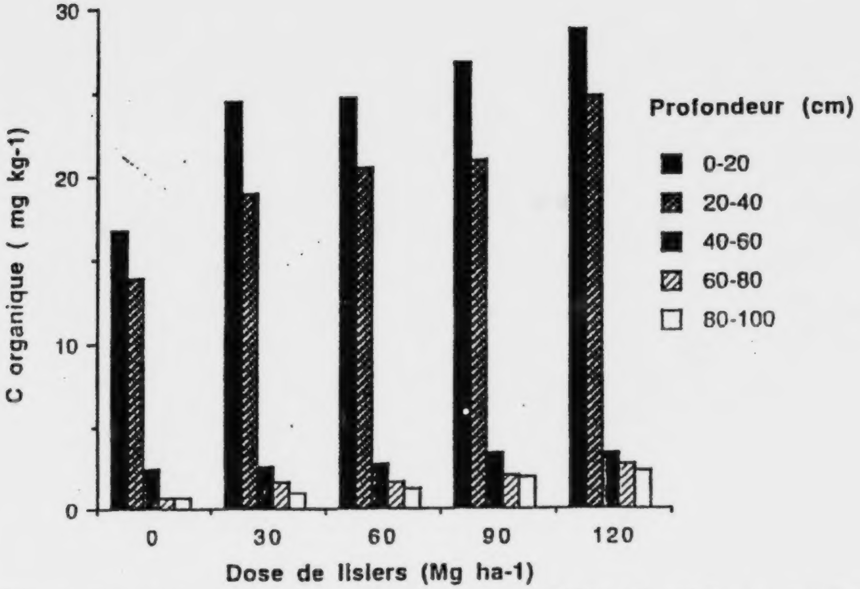

FIG. 1. Doses de lisiers et contenu en Corganique du profil de sol Neubois sous travail minimal

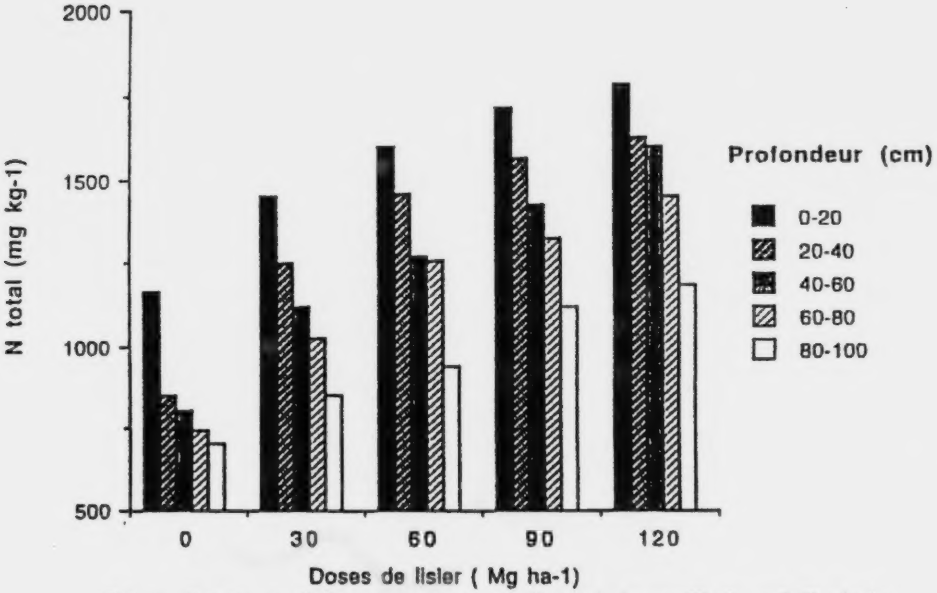

FIG. 2. Doses de lisier et contenu en N total du profil de sol Neubois sous travail minimal.

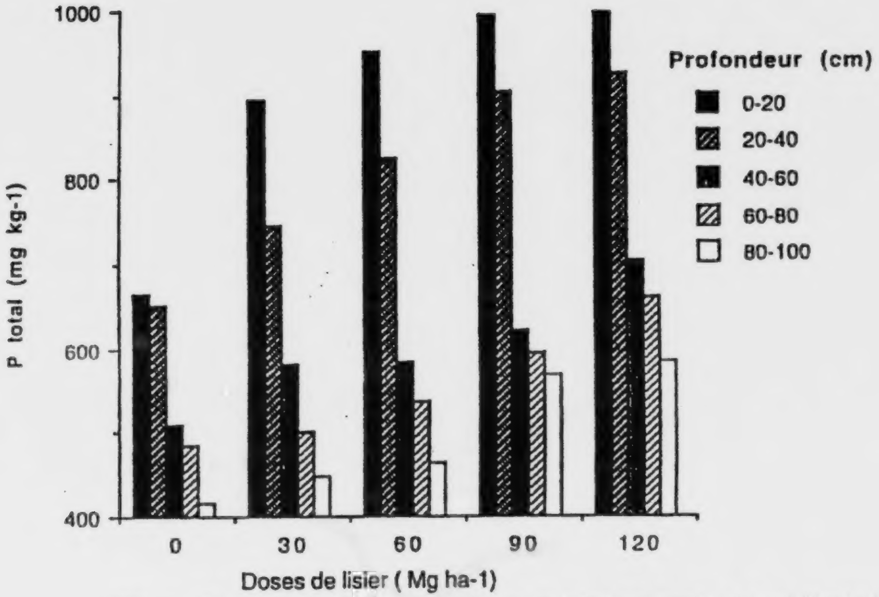

FIG. 3. Doses de lisier et contenu en P total du profil de sol Neubois sous travail minimal.

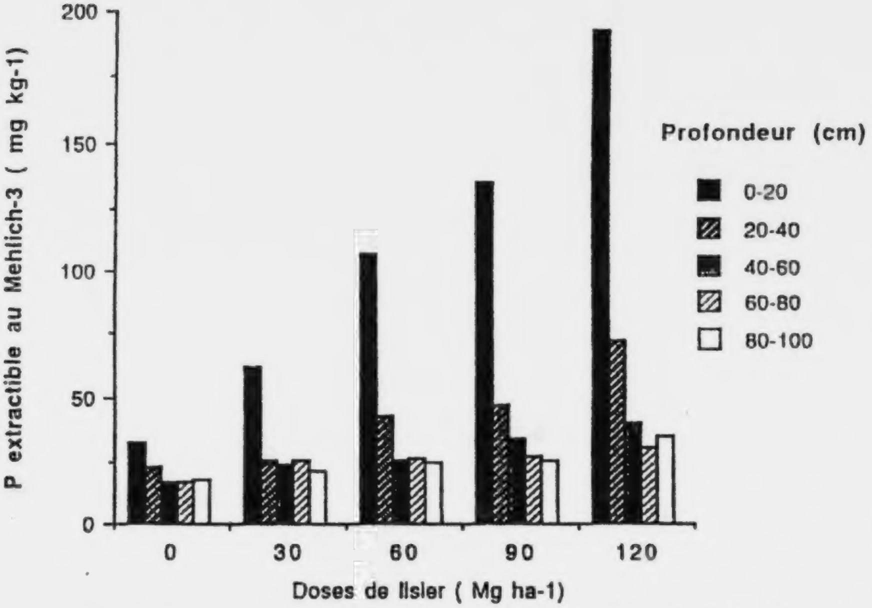

Fig. 4. Doses de lisier et contenu en P extractible du profil du sol sous travail minimal.

TRITURATES OF MACROPHYTES AND SETTLED PARTICULATES FOR BIOMONITORING PCB LEACHATE

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Sampling and analytical protocols were compared in this study. In the aqueous environment, PCBs partition to sediments of complex with colloids and other organic acids. They are absorbed by biota and any by other suspended materials which provide a hydrophobic surface for them to bind.

The site of interest is the St.Lawrence River (SLR) near the Akwesasne Mohawk Reservation. The General Motors Foundry has been depositing PCBs in landfills and lagoons since the opening of the Foundry in 1954. Reynolds Metals Corporation (RMC) is upriver and Aluminum Corporation of American (ALCOA) is six miles up the Grasse River, a tributary to the SLR. Aroclors 1248 was used extensively by each corporation and the aluminum factories generated polynuclear aromatic hydrocarbons (PAHs) and other aromatic hydrocarbons which may be co-extracted with the PCBs. Each corporation has state permits to continue discharging PCBs in their effluents.

The sampling methods compared were sediment, water and biota. The water sampling process is simplest, but the minimum detection limit is frequently less than that required. To avoid this, plants were sampled and PCBs water extracted from the accumulated materials (triturated). Thus, PCBs were desorbed from the plants and the settled materials and communities into water.

METHODS

Trituration begins with collecting a plant into a jar. After sampling, the water and all the particulate material is shaken from the plant in the closed jar in the laboratory, and the solution decanted off. The plants and the settled materials left from the first decantation are then subjected to another trituration with tap water and shaken and decanted again. The combined aqueous extracts are then filtered on glass fiber filters and the sediments, plants and waters extracted separately.

All of these samples were analyzed on GC using a calibration mixture of Mix Fr-1; 1:1:1:1: totalling 800 PPB of Aroclors 1016, 1221, 1254 and 1260. Aroclors 1248 congeners are fully represented by this calibration material as detected with GC/ECD. The MDL average per congener is 1 ng/L as shown by replicate extractions of spiked waters at a level of 100 ng/L Aroclors 1248 in acetone, with an equilibration time of less than one hour. Water samples show a typical preferential partitioning of the less chlorinated congeners to henerate an enrichment in the less substituted congeners such as peak 3, 22' and 26 chlorobiphenyl, which are represented in a much higher percentage than they are in a pattern of directly measured 1248.

Water samples were the simplest to analyze, requiring the least time and producing the least interferences. The samples from triturates had the advantage over water because PCBs in the colloid containing water producing were higher.

Use of sediment sampling involves either core samples or grab samples such as a clamshell dredge. Tissues analysis entails either fishing, or collection of snails by picking off the plants. Plants are sampled simply in a jar, as is water.

Extracting wet sediments may be done with sohxlet (up to a full day procedure) or sonicated (can be done in relatively few minutes). The organic solvents are back-extracted from the acetone-hexane-water mixture to remove the water-acetone fraction, leaving PCBs dissolved in hexane. Tissues need to be tissumized which is time consuming, or fish can be freeze dried and then digested with sulfuric acid to remove extraneous lipids while leaving the PCBs again in the hexane fraction. Plants are also tissumized, but simply triturating and liquid liquid extraction with hexane. The technique used for water is similarly very simple. Clean up for all of these techniques involves sulfur removal for sediments and tissues using elemental mercury or TBAHS (recommended by EPA) and then all are subjected to chromatographic separation with Florisil. The disadvantage for sediments and settled materials is the disruption and consequent release of heavily contaminated sediments to the water column. In a river, this means transport of suspect contaminated sediments further downriver. Variability between sediments is extremely high. Two sediment samples may be taken a few of inches apart on the benthic surface, and result in two completely different levels. Although both may be correct, they may not necessarily be representative of what levels are actually in the sediment. Fish are not easily captured, although they also represent an integrated exposure from their environment. However, if they are not in a primarily contaminated area all of their life, they will show lower levels of the hydrophobic toxins which illustrate their entire environmental exposure, and not show what levels would occur if they were raised in contaminated environment their entire lives.

Plants may not be present in an area of interest, however they are present in the areas of concern to us. Water sampling has the disadvantages of limited time windows as they are grab samples. The high volume of water required to gain a detection limit acceptable to regulatory agencies is prohibitive in terms of people hours involved in sampling 10 L to 100 L samples. Advantages to each of these techniques is that sediments have the highest PCB levels, and thus most easily detected levels. The tissues indicate the extent of potential human exposure due to the extent of biomagnification of contaminants through the food chain because people are going to eat the fish. Plants have the advantage of small sample size, as do snails. With the waters, the best advantage is that the analysis is extremely simple.

CONCLUSION

Long term monitoring requires an economical technique in order to establish the present concentrations and to establish the extent of bioavailability. Triturate samples were compared and overall show the same substantial enrichment in the lowly chlorinated congeners 2' and 26 chlorobiphenyls. Trituration overcome sampling variability by integrating river flux over time and spatial water flow, by concentrating the PCBs. Triturates also allow sampling with a small sample size, but still generate a highly contaminated matrix. Sample composites of this kind allow in minimal number of samples to be taken, which decreases the cost necessary to accomplish accurate analyses.

TOXICITY ASSESSMENT OF OIL COMPONENTS AND AN OIL TREATING AGENT USING THE *Photobacterium phosphoreum* BIOASSAY

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ABSTRACT

The Microtox® test, based on the bioluminescence inhibition of the marine bacterium *Photobacterium phosphoreum* was used to evaluate the oil toxicity in seawater. First, the toxicity of thee water soluble fraction (WSF) of a crude oil and its constituents such as aromatic and polyaromatic hydrocarbons was tested. The WSF toxicity was monitored while the evaporation and the photooxidation of oil components were in progress. In addition, the toxicity of various oil fractions formed in the marine environment after an oil spill (i.e. emulsified/degraded oil residues, surface film, dissolved components in the water column and settling oil residues) were tested for their potential toxicity over a 2 - month summer experiment conducted in 5 mesocosms (3.5m^3 each). A new anti-adhesive oil-treating agent designed for the treatment of oil slicks at sea was also tested in mesocosms together with the treated crude oil. These preliminary results show that the Microtox® test is very sensitive to oil compounds. Used in combination with mesocosms, it provides an efficient experimental tool to assess the toxicity of crude oil spilled at sea under natural conditions.

INTRODUCTION

The *Photobacterium bacterium* bioassay, well-known under the commercial name of Microtox® test, has been previously used to assess the effectiveness of treatments for oil process wastewaters (Peake & McClean, 1982; Delistraty, 1984; Jones et al., 1985; McTernan et al., 1986). However, in their exhaustive review of the toxicity of pure compounds, Kaiser and Ribo (1988) mentioned only a few data for some mono-aromatic hydrocarbons and naphthalene. In the course of our work on the fate and effects of crude oils spilled at sea, a wide experimental study has been conducted in our laboratory in Summer 1993 to collect fundamental data on the toxicity of crude oils and soluble hydrocarbons and further to evaluate the toxicity reduction following the natural oil degradation (Siron & Pelletier, this issue). In addition, this report presents a preliminary toxicity assessment conducted on new-designed oil-treating agent developed to fight oil spills at sea and improve clean-up operations (Pelletier & Siron, 1993). Because this study was exclusively conducted in seawater and regarding to the importance of the bacterial level in the degradation processes occurring in the marine environment, the luminescent bacterial bioassay a priori appeared particularly well adapted to this purpose. To our knowledge, the present report is the first one dealing with the application of the Microtox® test to crude oil. In this respect, some experimental protocols developed for oil testing are presented and the relevance to use the luminescent bacterial bioassay is discussed through these preliminary results.

Background

The bioassay using the light emitting marine bacteria *Photobacterium phosphoreum* as test organism has been developed commercially under the trademark Microtox® for about 10 years. Briefly, the test is based on the reduction of bioluminescence when bacteria are affected by xenobiotics accommodated in the tested medium. After transforming the dose-response curve in a log-log scale, results are expressed in

Effective Concentration (EC X) which represents the concentration of the toxicant showing a X % inhibition of luminescence relatively to the control population. The EC X can be expressed either in concentration of the tested compounds or in the percentage of volume of the toxic solution after dilution with uncontaminated water.

Test procedures

Tests were performed using the Microtox® model 500 Analyser (Microbics Co., Carlsbad, CA). Optimal physiological conditions are requested for bacterial reagent to give quality results (Ribo & Kaiser, 1987). The test temperature was automatically adjusted to 15 ± 0.5°C by the system, while the reconstituted reagent (freeze-dried bacteria) was maintained at 5.5 ± 1°C. Unless otherwise mentioned, the pH of seawater samples usually was in the optimal range (6-8). Because all experiments were performed with natural seawater pumped from the St.Lawrence Estuary (25-29°/$_{oo}$), no osmotic adjustment was needed and tested solutions were diluted with uncontaminated seawater. The bioluminescence was recorded after 5, 15 and 30-minute exposures for each sample. Data collection and reduction were made using the version 7.0 of the Microtox® software. The solide-phase test (SPT) procedure was used to test settling matter and solid residues. Known amounts of solids were suspended and mixed into clean natural seawater. The bacterial reagent was then exposed to the suspension for 20 min. at 15°C, prior to measure the bioluminescence. The SPT procedure measures the toxicity when bacteria are in direct contact with a suspension of particles, involving both the tested particles and compounds that may dissolve from the solid phase (Bulich et al., 1992).

Laboratory experiments

For laboratory measurements, the natural seawater was filtered on 0.22 µm, the pH was adjusted to 7.0 and then the seawater was autoclaved ("clean seawater"). Commercial hydrocarbons were solubilized in 1 or 2 L of clean seawater and standard solutions were tested within the following hours to minimize losses and degradation.

A stock water soluble fraction (WSF) of two crude oils (Forties and Western Sweet Blend) was formed by layering the crude oil on seawater (1:10) in a 4L glass bottle and then the seawater was gently stirred during 30h., allowing the soluble oil components to dissolve. The WSF was then collected after a 18h - decantation period (Siron et al., 1987). Several natural processes affecting oil compounds were studied for their effects on the WSF toxicity. The photooxidation of oil components present in solution was simulated by placing the WSF with an oil layer in front of white fluorescent tubes provided a light intensity of 10 000 lux. A similar experiment was repeated on the WSF without surface oil layer and subjected to a light intensity of 35 000 lux. Photooxidation tests were conducted at 3 ± 1°C to minimize the evaporation.

The evaporation process was simulated by means of two procedures: (i) A 1L. WSF sample was stirred in a 20cm diameter glass plate (large surface to volume ratio) placed under a fume hood; (ii) WSF samples were subjected to continuous nitrogen bubbling in 20mL flasks. Evaporation experiments were conducted at room temperature. While evaporation and photooxidation processes were in progress, the WSF was sampled at fixed times (1, 4, 6, 24, 48 and 96 hours) for toxicity testing. All glass equipment was sterilized to minimize the bacterial degradation of hydrocarbons during experiments.

A specific "contact-test" was developed to assess the potential acute toxicity of weathered oil residues, the so-called "tar-balls". Oil residues were naturally weathered in mesocosms, as described further in the section. An aliquot fraction (0.7g) of surface oil residues was placed in 20 mL clean seawater, shaked for 5 min. and kept in contact for 24h at 3°C in darkness. The contaminated seawater was then collected to record the Microtox® toxicity.

Mesocosm experiments

Mesocosm facilities of the INRS laboratory located at Pointe-au-Père (Québec) were used to test the toxicity of crude oil subjected to natural weathering processes. Mesocosms consist of stainless steel tanks ($3.5m^3$ each) that allow the sampling of surface water, water column and settling matter. Mesocosms and their specific devices are described elsewhere (Siron & Pelletier, this issue). The mesocosm experiment took place during summer and lasted 2 months. Tanks were filled with natural seawater pumped from the St.Lawrence Estuary. Four tanks were use in this experiment to test:

- 1) the crude oil (CO); Forties crude oil (350 mL) was spilled alone on the water surface;

- 2) a silicon-based oil treating agent (TA), (Pelletier & Siron, 1993); the treating agent (300 mL) was spilled on the water surface;

- 3) the treated crude oil (CO + TA); Forties crude oil (350 mL) was spilled and then the treating agent (300 mL) was applied to oil slicks. The fourth tank was used as control.

Water samples and oiled solid residues from surface, water column (1m depth) and sediment traps were collected over 2 2-month period in order to measure the overall toxicity of crude oil as a function of the natural weathering process at sea. Solid samples (i.e. floating residues (TA) and settling oiled residues) were dried (40°C for 24h) and tested using the SPT procedure. Because the toxicity measured with the SPT method basically results from a surface phenomena after a direct contact between bacteria and particles (Bulich et al., 1992), the contact area was improved by crushing solid samples to be tested into a fine powder prior to SPT measurements.

Data quality

A standard solution of phenol (100 mg/L) was used as reference to test protocols and to check the performance of the operator and the system (apparatus, reagent lots) in addition to establish the variability of the Microtox® test. Under our laboratory conditions, the coefficient of variation was maintained below 10% (average = 8%) throughout this study.

Toxicity of individual hydrocarbons

Aromatic hydrocarbons listed in Table 1 are major constituents of the water soluble fraction (WSF) of crude oils (McAuliffe, 1987; Siron et al., 1993). Basically, the solubility of these hydrocarbons gives a good indication of their relative distribution in the WSF. The toxicity of hydrocarbons (EC 50 values) measured with Microtox® was consistent over time (Table 1). Consequently, the EC 50 value read after 15 minutes was considered as representative of the mean toxicity for all tested compounds. Unless otherwise noted, this endpoint value has been considered in this study. For each hydrocarbon, the validity of results was supported by excellent dose-response relationships (R > 0.98). In addition, slopes of the linear regression ranged from 0.81 (2,3,5 trimethylnaphthalene) to 1.49 (xylene mixture) and 95% confidence ranges never exceeded 25% of the endpoint value. Microtox® data generally are in good agreement with lethal concentrations (LC 50) measured with other bioassays (see references cited in Table 1). In spite of the high variability of some LC 50 ranges reported in the literature, similar trends are known throughout the toxicity ranks of pure hydrocarbons. The EC 50 measured here generally are close to, or even below, the lower limit of LC 50 values reported in the literature (e.g. naphthalene, methylnaphthalenes, phenanthrene). For a comparison purpose, the n-hexane, a low-molecular weight saturated hydrocarbon was also tested.

Toxicity of the Water Soluble Fraction (WSF) of crude oils

The WSF generally may contain from 10 to 40 mg/L^{-1} of total oil extracts, mainly hydrocarbons, according to crude oils used and experimental conditions (Siron et al., 1987; McAuliffe, 1987). Basically, the chemical composition of the WSF is somewhat comparable when formed from similar crude oils (Shiu et al, 1990), allowing the comparison of results obtained with WSF of the two light crude oils used in this work. The EC X endpoint value was expressed in percentage of the tested volume (% volume) rather than in concentrations (mg/L) of toxic chemicals; for example, a 100% value corresponds to the indiluted sample. This unit seems to be more reliable when assessing the toxicity of crude oil of WSF over time because we are facing very complex mixtures of toxic compounds (as in industrial effluents) with chemical composition changing over time. Even if quantitative chemical analyses would be available, the EC X (expressed in concentration) would not refer to the same toxic compounds over time as the oil weathering was in progress. The EC 50 (or EC 10), when expressed in % volume, is considered as a measure of the total toxicity of the water sample, further in this report.

When the WSF was subjected to evaporation, the residual toxicity decreased over time (significant increases in EC 50 as shown in Fig. 1). It is noteworthy that the toxicity reduction occurred rapidly, within the first hours, whatever the experimental design was. For example, the WSF toxicity decreased from 1/2 to 3 times after 24 h of evaporation (Fig. 1). Then, the toxicity seemed to stabilize. When exposed to light, the WSF showed divergences in the toxicity following the exposure technique, either the WSF topped with an oil layer or the WSF surface kept free of oil (Fig. 2). Although not significantly different, the light seemed to induce an increase of the toxicity in the presence of an oil layer. In contrast, the WSF with free surface showed a decrease in the toxicity despites the higher light intensity involved (Fig. 2). In the latter case, particularly noticeable is the fact that the toxicity reduction seems to follow the pattern observed during the evaporation experiment (Fig. 1). The illuminates WSF however, had a toxicity of about 2 times higher than the evaporated WSF, after 96h. (The EC 50 stabilized around 15% for the illuminated sample).

Table 1: Toxicity of hydrocarbons forming the Water Soluble Fraction (WSF) of crude oils.

Hydrocarbons	Sol.$_{sw}$ (mg/L)	EC 50 (mg/L)			LC50 (mg/L) (literature)
		5 min.	15 min.	30 min.	
Hexane	75.5	71.2	70.8	60.0	3.5>100
Benzene	1780	31.1	40.1	47.2	5.8-127
Toluene	515	14.5	15.7	18.8	4.3-59
Xylene (o,m,p isomers)	175-198	3.3	3.8	4.0	1.3-24.6
Ethylbenzene	152	2.2	2.3	2.8	0.5-15.4
Naphthalene	20 ± 2	0.6	0.6	0.7	0.9-15
1-Methylnaphthalene	<20	0.3	0.4	0.5	0.7-9
2-Methylnaphthalene	<20	0.3	0.3	0.3	0.7-4.7
Dimethylnaphthalene (mixture of isomers)	2.4	0.2	0.3	0.3	0.08-5.1
2,3,5-Trimethylnaphthalene	1.7±0.6	0.8	0.8	0.7	0.3-2.0
Phenanthrene	0.6±0.1	0.2	0.3	0.3	0.4-0.7
2-Methylphenanthrene	0.3±0.1	0.4	0.3	0.3	0.3

Sol.$_{sw}$: Solubility in seawater (209-25°C). Data from Vershueren (1983) and McAuliffe (1987).

EC50: Effective concentration showing a 50% inhibition of bioluminescence after 5, 15 and 30 minutes (reading times based on the Microtox protocol).

LC50: Lethal concentration (50% mortality) from tests on various marine species over 24-96h. Data according to Vershueren (1983), Neff (1987) and McAuliffe (1987).

Toxicity of weathered and treated crude oil

The Microtox® toxicity of seawater collected at 1 m depth below the surface was monitored in the tanks which received the treating agent alone (TA), the crude oil alone (CO), and both of them (CO + TA). The toxicity remained at low levels throughout the experiment and EC 50 values exceeded 100% (undiluted sample) in most cases. For this reason, EC 10 values were calculated, allowing toxicity to be compared between tanks (Table 2). The EC 10 values indicated some low toxic effects of the contaminated seawater enclosed in tanks. The highest toxicity was observed early, within the first hours following the spill. At this moment, the toxicity increased from TA > CO > CO+TA.

The toxicity then decreased quickly, as revealed after the 24 h sampling. Unexpected low EC 10 values were measured after 7 days but this apparent toxicity disappeared later on.

The toxicity of the surface microlayer (i.e. the 500 μm thickness film) was also evaluated. The surface microlayer containing the oil sheen was found 3 to 5 times more toxic than the underlying seawater, including the control tank (Fig. 3). The surface microlayers from both "CO+TA" and "TA" tanks showed a toxicity slightly lower than the control and the "CO" tanks. In addition to the microlayer, floating treating agent and oiled residues were collected an their potential toxicity was tested. The contact-test revealed an acute toxicity of the unaltered crude oil at day 0 (EC 50 = 8% and EC 10 = 1%) and a subsequent decrease of toxicity over time for both treated and untreated residues (Fig. 4). No significant difference in the residual toxicity was noted when the crude oil was treated. The floating residues formed by the application of the treating agent alone were tested using the solide-phase test procedure (SPT). Either from laboratory or mesocosm experiments, the SPT revealed the very low toxicity of the treating agent when spilled alone (Table 3).

The Microtox® test was also applied to seawater and residues collected in sediment traps. As large amounts of solid residues were suspended in trapped seawater, two methods were used to discard the particulate matter and were tested for their effects on toxicity measurements (Table 4). As observed in the water column, the seawater enclosed in traps showed a relatively low toxicity. EC 10 values calculated for the trapped seawater are significantly different according to the separation procedures, for the three oil treatments tested (Table 4). For example, when centrifugated, both "CO+TA" and "TA" seawater samples were found more toxic. In contrast, the "CO" sample was found the most toxic when tested after a 24h- decantation period, whereas the centrifugation markedly reduced the toxicity.

Finally, the toxicity of settling matter recovered from sediment traps, including natural detritus mixed together with treating agent and oil residues, was directly tested as a solide phase (SPT). Because the seawater used as diluent became coloured after resuspending the settling matter, the colour correction protocol available with the Microtox® test was used. As displayed in Table 5, the colour of samples seems not to affect the SPT measurements. A marked gradient was found in the toxicity of the settling matter, form "TA" as the less toxic to "CO+TA" as the most toxic residues (Table 5). This toxicity gradient was in good agreement with that observed in the water column immediately after the contamination. Moreover, the toxicity of "TA" settling residues was quite comparable to that found for the floating residues, with regard to EC 50 values (Table 3).

Table 2. Toxicity of contaminated seawater during the mesocosm experiment.

TIME	CONTAMINANTS ADDED IN TANKS	EC 10 (% volume)	
		mean	95% C.R.
After 4 h	control	not toxic[a]	–
	treating agent (TA)	20.3*	10.3-40.2
	crude oil (CO)	8.6*	7.2-10.2
	treated oil (CO+TA)	5.0*	1.7-14.8
After 24 h	control	not toxic[a]	–
	TA	58.5*	40.7-83.9
	CO	32.4*	10.2->100
	CO+TA	32.1*	21.9-46.9
After 7 d:	control	16.0	9.0-28.3
	TA	17.1	11.8-24.7
	CO	14.0	6.0-33.0
	CO+TA	23.6	8.1-68.2
After 17 d:	natural seawater	>100	–
	CO	55.9*	31.3-99.6
	CO+TA	44.1*	28.0-69.4
After 57 d:	CO	49.8	39.3-63.0
	CO+TA	not toxic[a]	–

a: Microtox data displayed a bioluminescence enhancement relatively to the natural seawater used as diluent (negative slope for the dose-response linear regression);

*: Value significantly different from the control, with a 95% confidence range.

Table 3. Toxicity of solid residues of treating agent (TA) collected during the mesocosm experiment (Solid-Phase Test procedure).

SAMPLES	EC 50 (ppm)
Treating agent (2 laboratory tests)	81320 - 247860
Floating residues (mesocosms; day 1)	>100000
Floating residues (mesocosms; day 9)	34215 ± 13228
Wall-adsorbed res. (mesocosms; day 10)	58355 ± 23569

Table 4. Toxicity of the seawater collected from sediment traps (day 11).

Contaminants added in tanks	TSM (g/L)	EC 10 (% volume)			
		centrifugation		decantation	
		5 min	15 min	5 min	15 min
(control)	0.54	(a)	(a)	(a)	(a)
treating agent (TA)	4.00	26.3	27.2	55.7	(a)
crude oil (CO)	0.40	44.2	50.3	3.2	4.0
treated oil (CO+TA)	0.86	17.9	14.1	40.8	70.2

(a); Microtox data displayed a bioluminescence enhancement relatively to the natural seawater used as diluent.

Table 5. **Toxicity of residues collected in sediment traps after 11 days (Solid-Phase Test procedure).**

Contaminants added in tanks	EC 50 (ppm)	
	no correction	colour correction
treating agent (TA)	65310 ± 25119	55761 ± 18053
crude oil (CO)	2342 ± 470	2048 ± 513
treated oil (CO+TA)	333 ± 85	321 ± 82

DISCUSSION

Toxicity of soluble hydrocarbons

The comparison between Microtox® data and other toxicity data available in the literature (lethal concentrations) reveals that the bacterial luminescent bioassay is very sensitive to hydrocarbons, especially monoaromatic and polyaromatics. For example, the EC 50 measured for some di- and tri-aromatic hydrocarbons are the lowest reported to date. Soluble/volatile aromatic hydrocarbons are known to be the most toxic oil coponents to marine biota (McAuliffe, 1987). The Microtox® toxicity of aromatic hydrocarbons clearly increases with the molecular weight, indicating the relationship between toxicity and chemical structure of tested compounds. When comparing aromatic (benzene) and aliphatic (hexane) hydrocarbons with a similar molecular weight, the former appears about two times more toxic. In addition, the potential toxic effects of benzene and other mono-aromatics (BTEX) in the aquatic environment are reinforced by their higher solubility. Because EC 50 values measured for BTEX were much lower than their solubility concentrations reached in seawater below oil slicks, likely will exceed EC 50 values. Conversely, aromatic hydrocarbons with higher molecular weights showed EC 50 values very close to their solubility. For example, the 2-methylphenanthrene is the most toxic among tested compounds (EC 50 = 0.3 mg/L^{-1}), but little effects are expected under natural conditions of contamination (oil spills, petrochemical effluents(because its concentration in seawater will remain very low too (sol. = 0.3 mg/L^{-1}), and the EC 50 will not be reached easily in the aquatic environment.

The WSF of crude oils has been largely investigated with respect to its toxicity as well as its chemical composition. From this preliminary study conducted with the Microtox® test, toxicity results generally are in agreement with the oil chemistry. First, we took advantages of the fast bacterial response, and it was possible to run several tests within a 24 h. period while the volatilization of soluble hydrocarbons was simulated. It is particularly noticeable to observe that the curve displaying the toxicity reduction over time fits very well with oil evaporation curves (Mackay & McAuliffe, 1988). That provides some evidences that the reduction of oil toxicity was directly recorded as a function of the evaporation losses simulated in our experiments. This result was expected form the physical-chemical properties and toxicity of WSF hydrocarbons, because the more volatile/soluble hydrocarbons (mono-aromatics) are also toxic, confirming the evaporation as a major process that significantly reduces the oil toxicity at sea. The increased toxicity observed when the WSF was illuminated could be related to the photo-chemical reactions that occurred with some aromatic hydrocarbons dissolved in seawater (Ehrhardt et al, 1992). As the WSF toxicity declined when the crude oil layer was removed from the surface, even when exposed to more intense light, that might indicate either the photooxidized compounds are still volatile in spite of their increased polarity, or the illumination of the oil layer improved the solubilisation by forming more polar (oxidized) compounds, or both processes occurring at once. Here again, Microtox® data seemed to be in agreement with the chemical composition of the illuminated WSF (Ostgaard & Jensen, 1983; Siron et al, 1991), together with the increased toxicity of the photooxidized oil on bacterial activity (Larsson et al, 1979; Pengerud et al.; 1984).

Toxicity assessment of weathered/treated crude oil

The contact-test procedure developed to measure the potential toxicity of the so-called "tar-balls" provided a simple protocol in the preparation of samples to be tested. In the contact-test procedure, the oil-water ratio is adjusted to be comparable to the ratio used for form the WSF, allowing comparison between the toxicity of both fresh crude oil (WSF) and weathered oil residues (contact test). It was interesting to note that the toxicity of the undegraded crude oil (reference oil) was quite similar either by using the contact-test of the WSF procedures. In addition, the contact-test applied on floating oil residues confirmed the toxicity was decreasing over time, due to the natural oil weathering.

The surface microlayer testing confirmed the necessity of keeping a control medium when conducting an experimental toxicity assessment. Indeed, toxicity of the surface water was significantly higher than that measured at 1m depth, even in the uncontaminated natural seawater (control tank). Likewise, the water column sampled within the same period reflected a certain toxicity. In this respect, relatively high pH values (up to 8.2) were occasionally measured in seawater enclosed in mesocosms, whereas the same natural seawater with pH adjusted to 7 never showed any toxicity. That could explained such an unexpected toxicity, especially with the Microtox® bioassay which is very sensitive to some physical-chemical conditions (Ribo & kaiser, 1987).

In the water column of mesocosms, an acute toxic stress was recorded a few hours following the oil addition (oil spill). The toxicity actually declined very fast and remained low by the end of the experiment. This result was expected from toxicity measurements on volatile/soluble aromatic hydrocarbons. In addition, numerous weathering processes took place in mesocosms and the toxicity reduction of the spilled oil over time is in good agreement with changes in the chemical composition of various oil fractions (Siron et al., 1993; Siron & Pelletier, this issue). The discrepancy noted between the toxicity values of seawater collected in sediment traps could be related to the difficulty for partitioning trap samples containing high loads of suspended toxic particles. The Microtox® test likely was sensitive to both suspended residues and dissolved oil components, as detailed about the SPT procedure. The 24h - decantation period likely was not sufficient to make the trapped water clear of weathered oil residues which usually exhibit a density very close to seawater (Siron et al., 1993). In contrast, when the oil treating agent was tested (with of without crude oil), the significant increase in the toxicity of trapped seawater could reflect the centrifugation was not effective to separate suspended residues from trapped water. Both the treating agent and the fresh (undegraded) crude oil have a density lower than seawater, causing treated oil to float. As a result, the decantation was more efficient for recovering and testing the underlaying seawater free of particles, reducing the toxicity of the trap samples. This comparative study reveals that methods used to prepare water samples are crucial and could significantly affect Microtox® measurements. In contrast, tests on solid phase via the SPT procedure are more easy to manage, and revealed an unambiguously trend in the toxicity of residues collected in traps. The SPT protocol was sensitive enough to display significant differences in the toxicity of oiled residues, related to their chemical composition after various oil treatments. A synergistic toxic effect between the crude oil and the oil treating agent could actually be the result of the effectiveness of this new agent to fix the oil slicks into a solid matrix, avoiding the oil to be dispersed. By reducing the oil weathering, the treating agent causes the oil to be more toxic overt time when comparing to the untreated, but more weathered, oil residues. Although not statistically significant, the difference observed in the induced toxicity of floating residues after 2 months showed the same trend. In addition, solid residues formed by the application of the treating agent could be considered a little, if not, toxic, regarding to very high EC 50 values calculated for both floating and settling (traps) residues. The very low toxicity of these solid residues is reinforced by the fact that the SPT procedure is known to often display higher toxicity than does a basic test on the water obtained after elutriation or extraction of the solid phase (Bulich et al., 1992).

CONCLUSION / SUMMARY

As this preliminary study has proved the luminescent bacterial bioassay to be sensitive to crude oil and oil components, some specific protocols would be developed in future to better adapt various oil fractions formed in the aquatic environment to this bioassay. In addition, the rapid response of the Microtox® test was very useful to measure short-term (i.e. < 24h) changes in the toxicity. This point is particularly important in the toxicity assessment of pollutants undergoing physical-chemical alterations. In this respect, the fast evolution of toxicity when oil components are subjected to weathering processes highlights the Microtox® bioassay as a well adapted approach to the toxicity assessment of the oil pollution in the aquatic environment. In the other hand, as the ultimate fate of spilled oil, settling oil residues and oiled sediments can be tested using the SPT procedure. The direct toxicity measurement on the solid phase appeared more reliable than the basic test on water when high amounts of oiled residues are suspended (> 0.4 g/L), making the water sample difficult to measure and decreasing the reproductibility of the Microtox® response.

Finally, the effectiveness of the combination between the Microtox® test (micro-scale bioassay) and the mesocosm facilities (macro-scale simulation) to provide an experimental tool for comparing the potential toxicity of various oil treatments and oil-treating agents was demonstrated through this study.

ACKNOWLEDGEMENTS

The authors wish to thank Caroline Piché for their technical assistance. This study was funded by ne NSERC through the Strategic Grant Program (STRGP 245).

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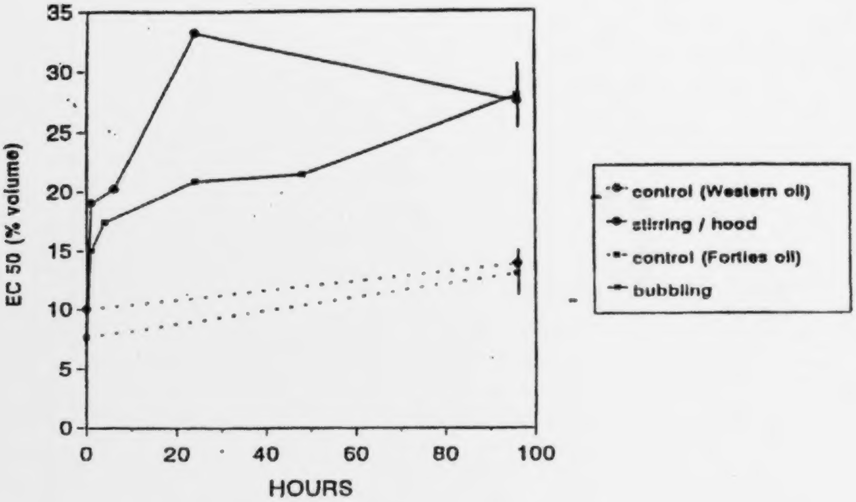

FIGURE 1: Toxicity of WSF when undergoing a continuous evaporation over time

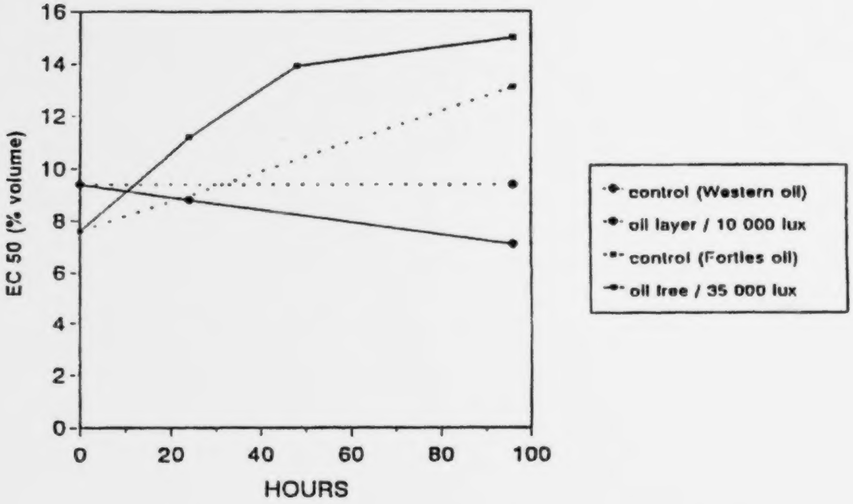

FIGURE 2: Toxicity of WSF when undergoing a continuous illumination over time

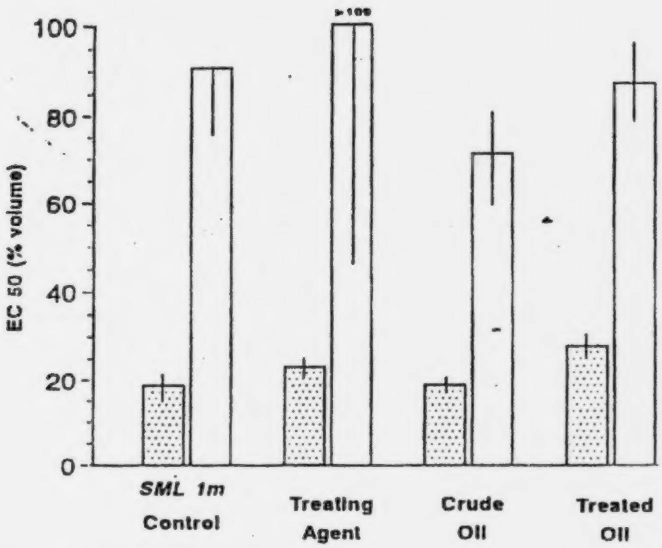

FIGURE 3: Toxicity of the surface microlayer (mesocosms; 9 days)

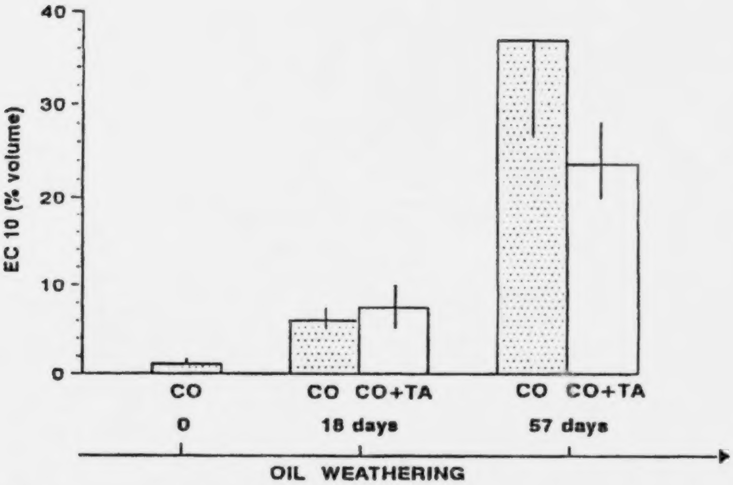

FIGURE 4: Toxicity of floating oil residues (contact test)

AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF CHLORINATED WASTEWATER EFFLUENT DISCHARGES FROM FOUR SEWAGE TREATMENT FACILITIES IN THE ATLANTIC REGION

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ABSTRACT

The final effluent from four sewage treatment facilities was chemically characterized and evaluated for acute and sub-lethal aquatic toxicity. The impact of chlorinated effluent discharges on the benthic macroinvertebrate community was determined at two treatment facilities discharging to a marine environment. GC/MS screening identified a wide variety of non-chlorinated compounds and some phenolic chlorinated compounds in effluents. The acute toxicity of the effluent to rainbow trout (*Oncorhynchus mykiss*), threespine stickleback (*Gasterosteus aculeatus*), *Daphnia magna* and *Photobacterium phosphoreum* was more pronounced with effluent from plants which had mean total residual chlorine concentrations above 0.2 mg/L. Abnormal shell development in larval oysters (*Crassostrea gigas*) was observed at concentrations as low as 0.2% after a 48-h exposure to effluent from one plant. All effluents tested were not toxic to the alga *Selenastrum capricornutum* and the cladoceran *Ceriodaphnia dubia* and were not mutagenic. A statistically significant decrease in the diversity of benthic macroinvertebrates was observed at sampling stations less than one metre from the effluent outfall of all plants and at 50 metres downstream of the outfall of one plant discharging to a brook. It was not possible to determine whether residual chlorine or other substances contributed to the observed effects on benthic macroinvertebrate diversity at the three sites.

INTRODUCTION

Chlorine is widely used for the control of various bacteriological and viral pests in municipal wastewater treatment in the Atlantic provinces. A 1990 use pattern assessment of disinfectant chemical use in wastewater treatment in the Atlantic provinces indicated that more than 672,000 kg of chlorine gas was used annually (Garron 1992). In addition to being an effective bactericide, chlorine has other benefits which include colour removal, taste and odour control, suppression of algal growth and precipitation of iron and manganese (CCREM 1987).

Municipal wastewater effluents contain many chlorinated compounds which result from complex reactions between chlorine added during wastewater treatment, and the organic material already present in the wastewaters. Some of those compounds are known to pose potential health risks while the residual chlorine in the wastewaters may be acutely lethal to fish and other aquatic life. While the acute toxicity of municipal wastewater effluents has been well documented (Environment Canada 1978; OMOE 1990), measured impacts on aquatic ecosystems, particularly those in the Atlantic provinces, have not been well established.

The objectives of this study were:

- to chemically characterize final effluents from sewage treatment facilities which use chlorine for disinfection;
- to determine the acute and sub-lethal toxicity of chlorinated wastewater effluents to a variety of aquatic organisms; and

- to measure changes in the structure of benthic macroinvertebrate communities related to chlorinated wastewater effluent discharges.

MATERIALS AND METHODS

Study Sites

Four sewage treatment facilities in Nova Scotia that used chlorine for disinfection were studied (Figure 1). Two of the facilities discharged to freshwater riverine environments and two of the plants discharged to marine environments.

The Greenwood Sewage Treatment Plant (STP) discharged to Fales Brook. It was a secondary treatment plant and the smallest one surveyed. It was designed to treat 800 m^3/day (Environment Canada unpublished data) but was receiving over 1,100 m^3/day during the survey (K. Redden, Municipality of Kings Co., pers. comm.). To compensate for the fact that the plant was hydraulically overloaded, the plant overchlorinated to ensure adequate removal of pathogens.

The Lakeside STP was a tertiary treatment system that was designed to treat 4,500 m^3/day (Environment Canada unpublished data) but was treating only 1,350 m^3/day during the survey (W. Collins, Municipality of Halifax Co., pers. comm.). The plant discharged to Nine Mile Brook.

The CFB Cornwallis STP discharged to the Annapolis Basin, a marine area with extreme tides. It used secondary treatment technology and was designed to treat 2,250 m^3/day (Environment Canada unpublished data).

The Eastern Passage STP was a primary treatment plant and the largest one surveyed. It was designed to treat 17,275 m^3/day (Environment Canada unpublished data) and discharged to Halifax Harbour.

Effluent Collection

Final effluent samples were collected for chemical characterization and toxicity assessment from the four STPs surveyed during the fall of 1991. A 24 h final effluent sample was collected from each plant, with an additional sample collected at CFB Cornwallis at a later date.

Effluent samples were collected with Sigmamotor Model 6200 automatic samplers. To minimize the potential for contamination from the medical grade silicone rubber tubing used in the pump head, lengths of silicone tubing were restricted to 30 cm.

For the samples for chemical characterization (excluding metals), a uniform volume of effluent was collected every 15 minutes over a 24 h period in a 20 L glass carboy that had been detergent washed and rinsed with distilled water, acetone and hexane. Stainless steel tubing (1 cm ID) was used to transport the effluent to the automatic sampler and from the sampler to the carboy. The composite sample was kept on ice during the collection period. After the 24 h period, the composite sample was vigorously mixed then subdivided into appropriate containers. The subsamples were transported to the Environmental Protection Atlantic Region lab on ice and were preserved in accordance with EP Atlantic Region's established protocol.

For the samples for toxicity assessment and metals analyses, a uniform volume of effluent was collected every 15 minutes over a 24 h period in a 208 L heavy duty, high density polyethylene drum, that had been detergent washed and rinsed with distilled water. Food grade, vinyl tubing (8 mm ID) was used to transport the effluent to the automatic sampler and from the sampler to the drum. After the 24 h period, the composite sample was vigorously mixed and a subsample was collected for metal analyses. Then eight 20 L food grade, polyethylene buckets were filled for toxicity assessment using rainbow trout, *Daphnia magna* and Microtox. Those samples were transported to the EP lab within two hours of collection. Four 4 L amber glass bottles were filled for *Ceriodaphnia dubia* (2 bottles), Ames and *Selenastrum capricornutum* tests (1 bottle) and bivalve larvae bioassays (1 bottle). Those subsamples were shipped on ice to three private laboratories for testing within 24 hours of collection.

Chemical Analyses

Base/neutral and acidic semivolatile extractables in effluent samples were identified by capillary gas chromatography/mass spectrometry (GC/MS) using computerized mass spectral search techniques. One litre of sample effluent was adjusted to pH 11.0 with NaOH and extracted with methylene chloride to obtain a base/neutral fraction. The pH of the effluent was then adjusted to 2.0 with sulphuric acid and extracted with methylene chloride to obtain an acidic fraction. Both fractions were dried over anhydrous sodium sulphate and concentrated separately to a small volume. Analysis was conducted on a Hewlett Packard 5890 gas chromatograph coupled to a Hewlett Packard 5971 Mass Selective Detector. The analytical column was a DB-5 30 meter phenylmethyl crosslinked fused silica capillary column. The gas chromatograph was temperature programmed initially at 40°C for four minutes, then 10°C/minute to 280°C and held for 20 minutes. The mass spectrometer was operated in full scan mode (EI. 70 ev). The scan range was 35 to 500 AMU with a scan speed of 0.92 cps. Semivolatile organic compounds in the effluent samples were tentatively identified by computer probability matching of unknown sample mass spectra with reference spectra contained in a Wiley 130000 mass spectra library. Only those compounds with a high computer generated match probability (>70%) were reported.

Tissue samples from mussels and freshwater clams held in the receiving environments were analyzed for underivatized di- and trichlorophenols by high resolution GC-ECD (DB-5 30 m fused silica). Approximately 10 g of homogenized tissue was weighed and deposited into a glass beaker, spiked with a 2,4,6-tribromophenol recovery surrogate and acidified with phosphoric acid. The samples were blended with anhydrous sodium and solvent extracted with methylene chloride using a Polytron tissumizer at high speed. Lipid in the solvent extracts was removed by GPC size exclusion chromatography and the extracts further purified by extracting phenolic compounds into aqueous 2% potassium carbonate, extracting several times with hexane, adjusting the pH of the solution to 2.0 and back-extracting phenolics into methylene chloride. The whole procedure afforded mean recoveries (n=3), from spiked matrix samples, of 41±6.5% to 86±15% for the dichlorophenols and 66±4.9% to 97±6.9% for the trichlorinated phenols.

Effluent samples for metal analyses were preserved in the field with 2 mL/L nitric acid and were analyzed for total metals under standard operating conditions using a simultaneous Inductively Coupled Plasma (ICP) instrumentation. The instrument used was the Thermo Jarrell-Ash Model ICAP-61E. All chemical analyses including: pH, alkalinity as $CaCO_3$ by inflection point titration (using a pH meter); Biochemical Oxygen Demand (BOD); Chemical Oxygen Demand (COD); Suspended Solids (SS); ammonia, using specific ion electrode; and Total Residual Chlorine (TRC), using an amperometric titrator, followed the recommended methods prescribed in American Public Health Association et al. (1989).

Toxicity Assessment

Rainbow trout (*Onchorhynchus mykiss*), threespine stickleback (*Gasterosteus aculeatus*) and *Daphnia magna* bioassays were conducted by the EP Atlantic Region lab and were conducted in conformance to the appropriate Environment Canada test method (Environment Canada 1990a; Environment Canada 1990b; Environment Canada 1990c). Freeze-dried luminescent bacteria (*Photobacterium phosphoreum*) were used to test effluents using the Microtox Toxicity Assessment System (Microbics 1989). For those effluent samples which contained greater than 50 ppb of residual chlorine, a second test was run simultaneously whereby the chlorine was neutralized with the minimum necessary amount of sodium thiosulphate prior to adding fish, daphnid neonates or bacteria.

Samples were also tested for toxicity using the algal (*Selenastrum capricornutum*) test for effects on growth and survival, the *Ceriodaphnia dubia* test for effects on survival and reproduction, the bivalve larvae bioassay using *Crassostrea gigas* for effects on larval growth and development, and the Ames test for mutagenicity. The *Selenastrum capricornutum* test was performed according to the methods of Green et al. (1988). Three brood *Ceriodaphnia dubia* survival and reproduction tests were performed according to the U.S. EPA method (Weber et al. 1989). Bivalve larvae bioassays using the Pacific oyster, *Crassostrea gigas*, were conducted according to the procedures described by ASTM (1989). The Ames test was performed according to the methods of Maron and Ames (1983). Four distinct mutant strains of *Salmonella typhimurium* (TA97, TA98, TA100 and TA102) were used and two variations of the test were performed (the spot test and the plate incorporation test).

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate abundance was determined at four stations (one upstream control, three downstream stations) in Fales Brook (Greenwood STP) and Nine Mile Brook (Lakeside STP) using gravel bag artificial substrate samplers. The samplers were constructed of 2 mm washed crushed stone in 1 mm knotless nylon mesh enclosures (15 x 10 x 3 cm). Six samplers were deployed at each station. Stations had similar bottom types, depth of water (25 to 50 cm) and proximity to shore. Where possible, samplers were deployed in the visible effluent plume.

Samplers were retrieved at least one month after being deployed, a time period observed to be suitable for colonization by endemic benthic macroinvertebrates (Coleman and Hynes 1970). Samples were collected using a 710 μm mesh sieve and placed in a one litre container with sufficient isopropanol to cover the sample. The invertebrates were sorted by hand under a dissecting microscope and identified to the lowest convenient taxon and enumerated.

Benthic macroinvertebrate abundance was determined at four stations (one control, three impacted stations) in Halifax Harbour (Eastern Passage STP) using a Peterson benthic grab sampler. Three grab samples from an area of a approximately 930 cm^2 each, were collected at each station and deposited into a large container. Four random subsamples were collected from the composite sample using a large spoon and deposited into one litre mason jars. The jars were three quarter filled with sediment and topped up with isopropanol and well shaken. The invertebrates were sieved, sorted by hand under a dissecting microscope and identified to the lowest convenient taxon and enumerated.

Caged Biota

In order to measure di- and trichlorophenols in the receiving environments, ten caged freshwater clams (*Anodonta spp.*) or ten blue mussels (*Mytilus edulis*) were deployed at each of the four sampling stations during the benthic macroinvertebrate surveys. The biota were held in stainless steel cages that had been detergent washed and rinsed with distilled water, acetone and hexane. Surviving biota were collected one month after being deployed, wrapped in aluminum foil that had been rinsed with acetone and hexane, and frozen until organic analyses could be conducted.

RESULTS AND DISCUSSION

Tables 1 to 4 present the base/neutral and acidic semivolatile extractables identified by GC/MS screening of the effluent samples from the four STPs surveyed. All of those compounds have a computer generated match probability of greater than 70 percent.

Generally, the organic compounds identified in the effluent samples fell into the following six chemical groups:

- detergents (e.g. 2-butoxy ethanol);
- solvents (e.g. alkylated benzenes, trimethyl pentanediol);
- fecal derived compounds (e.g. dihydrocholesterol);
- chlorinated compounds (e.g. dichlorophenols, dichlorobenzoic acids);
- plasticizers (e.g. bis(2-ethylhexyl) benzene dicarboxylic acid ester);
- miscellaneous compounds (e.g. caffeine, nicotine).

Some of the compounds identified in the effluent samples have been previously identified as representative chemicals of chlorinated wastewater effluents. Those organic compounds range from trihalomethanes to complex chloroaromatics, as well as chlorinated purines and pyrimidines (Glaze and Henderson 1975; Environment Canada 1978).

Chlorinated phenols, identified in the base/neutral screening of the effluent samples, were not detected in any of the clam or mussel samples held in the receiving environments. The following chlorinated phenols (with detection limits in ug/g d.w.) were not detected in the tissue samples collected; 2,3-dichlorophenol (<0.09); 2,4/2,5-dichlorophenol (<0.09); 2,6-dichlorophenol (<0.09); 3,4-dichlorophenol (<0.09); 3,5-dichlorophenol (<0.15);
2,3,4-trichlorophenol (<0.05); 2,3,5-trichlorophenol (<0.05); 2,3,6-trichlorophenol (<0.05); 2,4,5-trichlorophenol (<0.05); and 2,4,6-trichlorophenol (<0.05).

TABLE 1 - GC/MS EFFLUENT CHARACTERIZATION - EASTERN PASSAGE STP

2-butoxy ethanol [71]
ethylmethyl benzene [79]
trimethylbenzene [93]
benzenemethanol [86]
methyl(methylethyl)benzenes [76-96]
benzeneethanol [93]
tetramethylbenzene [88]

TABLE 1 (cont'd)
GC/MS EFFLUENT CHARACTERIZATION - EASTERN PASSAGE STP

dimethylethylbenzene [86]
methyl-(methylethyl)cyclohexanol [89]
methyl-(methylethyl)cyclohexen-1-ol [86]
napthalene [97]
2-(2-butoxyethoxy)ethanol [93]
alpha-terpineol [81]
dimethyl-(methylethyl)benzene [71]
phenoxyethanol [79]
diacetin [70]
3-(1-methyl-2-pyrrolidinyl)pyridine (nicotine) [95]
dodecanol [89]
(1,1'-biphenyl)-2-ol [92]
N,N-diethyl-methylbenzamide [95]
1,2-benzenedicarboxilic acid, diethyl ether [95]
cyclododecane [95]
hexadecanol [96]
caffeine [99]
cyclohexadecane [96]
hexadecanoic acid [83]
1,2-benzenedicarboxylic acid dibutyl ester [95]
sulphur [79]
2-butyl-phospate ethanol [81]
1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester [79]
androsterone [93]
ergost-5-en-3-ol [91]
choles-5-en-3-ol [98]
hexanoic acid [81]
methylphenol [94]
2-(2-butoxyethoxy)-ethanol [83]
benzeneacetic acid [89]
nonanoic acid [83]
methylbenzoic acid [83]
hydroxybenzoic acid [94]
benzenepropionic acid [93]
decanoic acid [96]
hydroxybenzoic acid methyl ester [95]
dichlorobenzoic acid [96]
dodecanoic acid [86]
tetradecanoic acid [94]
tetradecanol [88]
hexadecenoic acid [94]

[ ] computer generated match probability

TABLE 2 - GC/MS EFFLUENT CHARACTERIZATION - GREENWOOD STP

2-ethyl-1-hexanol [88]
trimethylpentanediol [81]
3-(1-methyl)-2-pyrrolidinyl) pyridine [95]
2-methyl-3-hydroxy-2,4-trimethyl propanoic acid [79]
N,N-diethyl-3-methyl benzamide [93]
2-(methylthio)benzothiazole [89]
1-methyl-5-(3-pyridinyl)-2-pyrrolidinone [83]
6-chloro-N-ethyl-N'isopropyl-1,3,5-triazine-2,4-diamin (atrazine) [99]
caffeine [97]
hexadecanoic acid [71]
hexadecanol [88]

Figure 1 - Study Sites

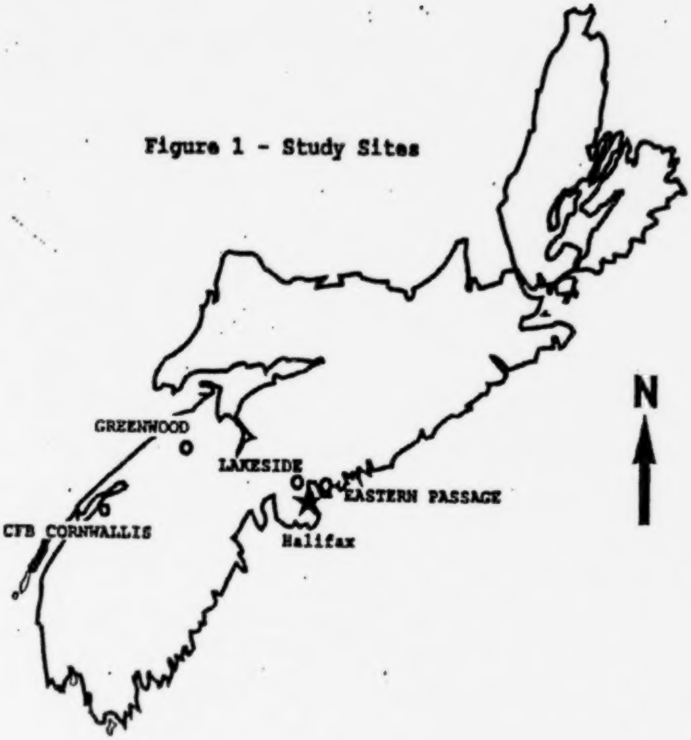

N
GREENWOOD
LAKESIDE
EASTERN PASSAGE
CFB CORNWALLIS
Halifax

Figure 2
Toxicity of Chlorinated Wastewater Effluent from Four Sewage Treatment Plants

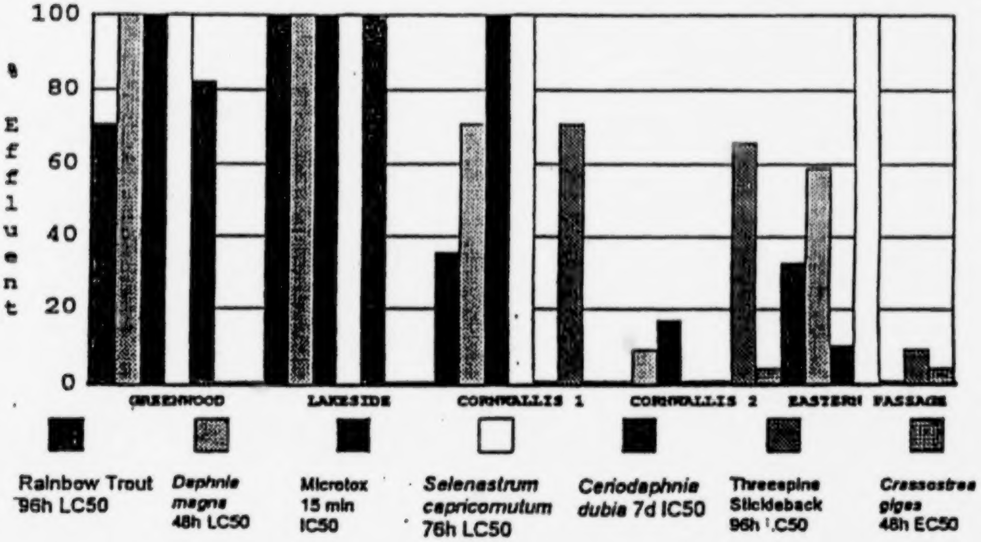


Figure 3
Toxicity of Chlorinated Wastewater Effluent from Four Sewage Treatment Plants Before and After Chlorine Neutralization

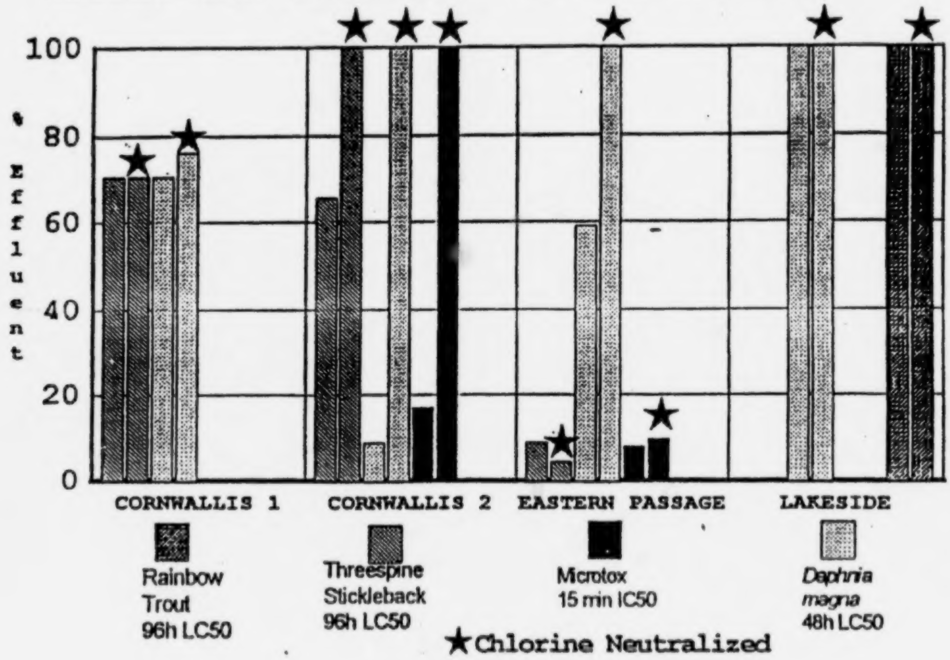


Figure 4
Mean Number of Taxa Collected at Sampling Stations at Three Sewage Treatment Plants

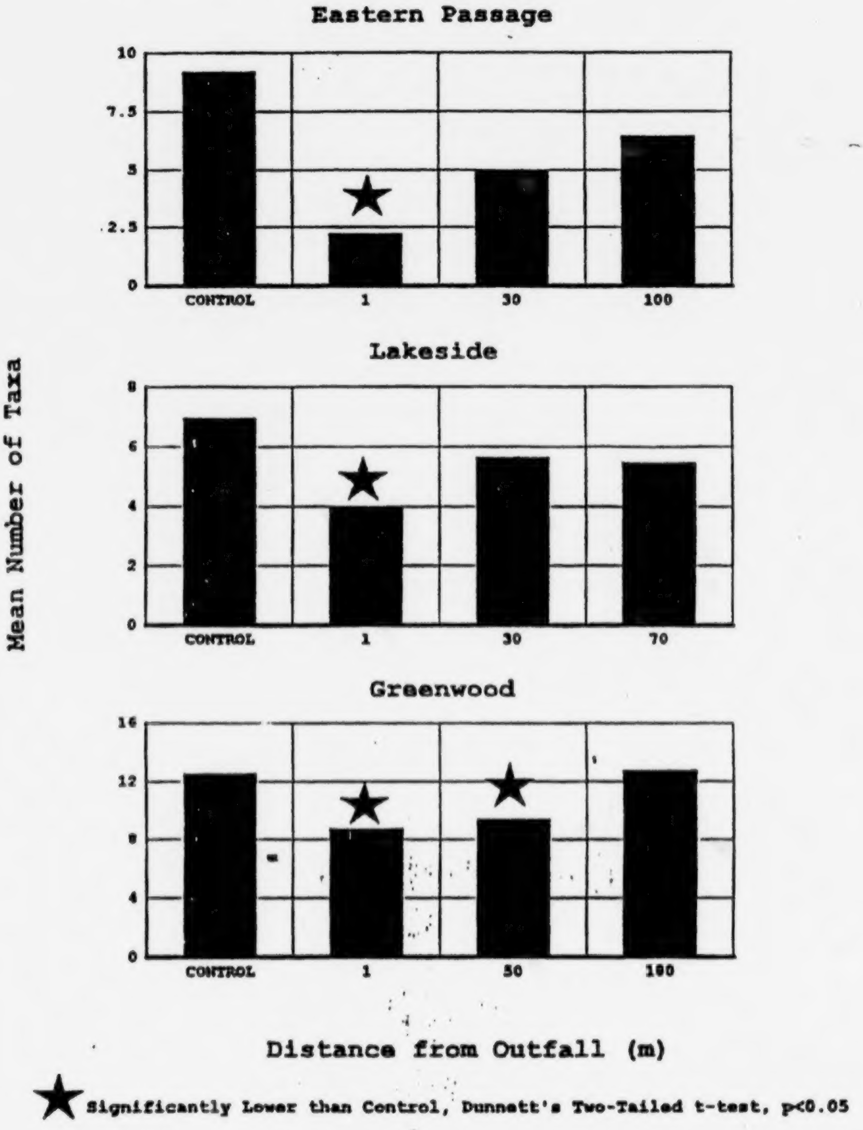
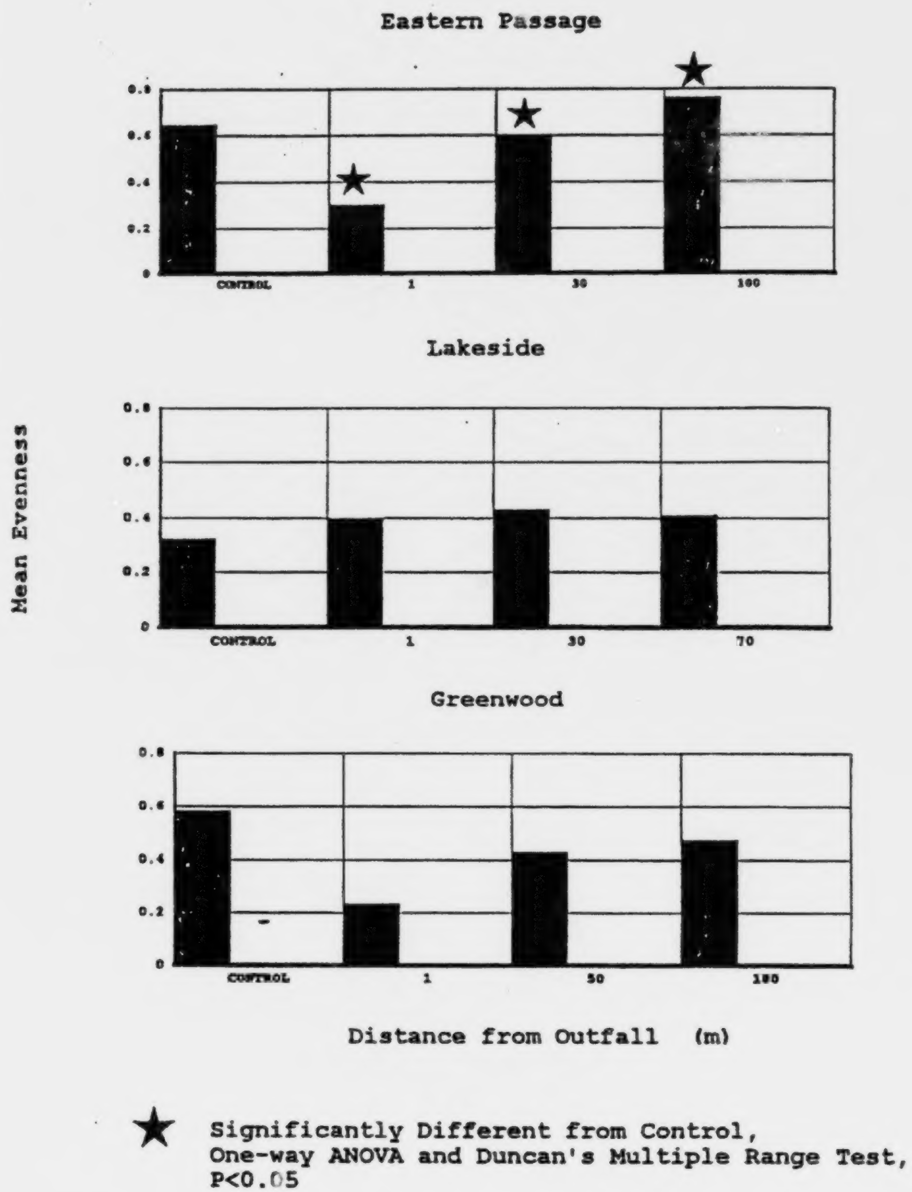


Figure 5
Mean Evenness of Benthic Taxa at Sampling Stations at Three Sewage Treatment Plants


Eastern Passage

Lakeside

Greenwood

★ Significantly Different from Control, One-way ANOVA and Duncan's Multiple Range Test, $P<0.05$

Table 5
Analytical Results for Selected Contami
from Four Sewage Treatment Plants (mg/L

Parameter	Cornwallis 1	Cornwallis
NH_3	<0.14	7.2
TRC	0.27	0.85
BOD	<5.0	<10.0
SS	45.0	34.0
pH	4.8	6.9
Al	11.51	1.8
Cu	0.12	0.05

nants in Final Effluent Samples
)

2	E.Passage	Greenwood	Lakeside	Federal Guidelines
	18.0	28.0	2.0	-
	0.25	0.005	0.12	1.00
	80.0	50.0	<5.0	20.0
	31.0	25.0	12.0	25.0
	8.0	8.2	7.8	6-9
	0.32	0.28	0.34	-
	0.02	0.02	0.03	-

ACUTE TOXICITY OF OIL SANDS WASTEWATER: A TOXIC BALANCE.

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ABSTRACT

The goals of this study were 1) to identify and determine the relative importance of the acutely toxic fractions of wastewater from oil sands extraction using a bioassay directed Toxicity Identification Evaluation (TIE) and to use these data to construct a toxic balance, and 2) to determine whether the same fractions were acutely toxic to Microtox, *Daphnia* and rainbow trout. Samples of extraction wastewater were obtained from the Mildred Lake tailings pond at Syncrude Canada Ltd. during the summers of 1991 and 1992. The samples were centrifuged to remove suspended solids and the toxicity of the supernatant was evaluated. Seven manipulations, each of which was designed to remove a different class of compounds, was performed on the supernatant samples. A complete TIE was performed using the Microtox bacterial bioassay. All acute toxicity of the surface tailings pond water to Microtox was removed by precipitation of organic acids or by removal of nonpolar organics. These results suggest that the main toxic fraction was a surfactant. In the interstitial water of the fine tails, volatiles accounted for 15% of the acute toxicity. However, all the acute toxicity of interstitial water was removed by precipitation of organic acids or by removal of nonpolar organics. Organic acids accounted for all the acute toxicity of tailings pond water to *Daphnia* and rainbow trout. Differences in relative sensitivity of test organisms to the toxic fraction show the importance of using more than one test organism to evaluate acute toxicity (*Daphnia* was 0.4 times less sensitive and rainbow trout were 3 times more sensitive than Microtox).

INTRODUCTION

An efficient way to identify and determine the relative importance of various classes of acutely toxic compounds in a complex mixture is to use a Toxicity Identification and Evaluation (TIE) protocol such as developed by the U.S. Environmental Protection Agency (EPA, 1991). The main advantage of the TIE methodology is the use of bioassays to identify the general toxicants in a complex mixture thereby allowing for the focussed analytical identification of the specific toxicants or the treatment of the toxic fractions without the costly attempt to identify the specific toxicants. The goals of this work were to identify and determine the relative importance of the acutely toxic fractions of oil sands extraction wastewater from Syncrude Canada Ltd. using a bioassay directed Toxicity Identification Evaluation (TIE) and to use these data to construct a toxic balance, and 2) to determine whether the same fractions were acutely toxic to Microtox, *Daphnia* and rainbow trout.

Syncrude Canada Ltd., located in northeastern Alberta, Canada, separates oil from oil sand using the hot water flotation method, which results in the production of large volumes of fluid wastes (process-affected water and a relatively stable suspension of solids and unrecovered bitumen called fine tails) (MacKinnon, 1989) which are acutely toxic to aquatic organisms (MacKinnon and Retallack, 1982; MacKinnon and Boerger, 1986). This fluid waste, which is currently stored in large tailings ponds on the Syncrude lease site, must eventually be reclaimed (Boerger et al. 1992). While the acute equal reductions in acute toxicity occurred whether samples were sparged with oxygen or nitrogen it can be concluded that both manipulations were removing volatiles rather than oxidizable compounds from solution. Approximately 15% of the acute toxicity of fine tails interstitial water can be attributed to volatile compounds (Table 4). The volatiles removed by these manipulations must also be non-polar organic compounds as they were removed by the C_{18} column. This is consistent with the effluent discharge to the ponds. The tailings pond at Syncrude receives low molecular weight hydrocarbons (volatile non-polar organic compounds) that are used as diluent in the bitumen froth treatment and not recovered by the diluent recovery system (MacKinnon and Sethi, 1993). These light hydrocarbons enter the pond as a slurry, so that some is lost to the atmosphere and the remainder is trapped in the fine tails (MacKinnon and Sethi, 1993). This is supportive of our result that acutely toxic volatile organic compounds were present in fine tails interstitial water but not in surface tailings pond water. However, there is also an apparent contradiction in these data. All the acute toxicity of the interstitial water was removed with the precipitation of organic acids at pH 2.5 and some (~15%) was removed with the removal of volatiles, yet most organic acids are not known for their volatility. It is possible that the removal of volatile compounds in the treatment to remove organic acids was an artifact of centrifugation.

While the bioassay results with *Daphnia* and rainbow trout paralleled those for Microtox for the reductions in acute toxicity with sample treatment, the sensitivity of each organism to surface tailings pond water differed dramatically. Rainbow trout were approximately three times more sensitive than Microtox and approximately seven times more sensitive than *Daphnia* to the acutely toxic compounds found in surface tailings pond water. Conversely, *Daphnia* were approximately 0.4 times less sensitive than Microtox to the acute toxicity of surface tailings pond water. These results demonstrate that the acute toxicity of wastewaters should not be tested with Microtox alone. At the very minimum, Microtox results indicating no acute toxicity should be validated with bioassays using other test organisms such as *Daphnia* and rainbow trout.

ACKNOWLEDGEMENTS

The authors would like to thank all our colleagues for the technical support that has aided in the successful completion of this study. A special thanks to Munir Jivraj for his diligent assistance in running the bioassays. This work was funded by the Fine Tailings Fundamentals Consortium with Alberta Oil Sands Technology and Research Authority as the operator under agreement # 8878M. The work was also facilitated through the cooperation of Syncrude Canada Ltd. in the form of providing samples as well as logistical support. The authors are grateful to Syncrude for permission to publish this research.

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Table 1: Results for the manipulations of the Toxicity Identification Evaluation (TIE) that did not result in a reduction of the acute toxicity of surface tailings pond water assessed using the Microtox assay.

#	TIE manipulations	pH	IC_{50} (mL/L) $\bar{x}$ (replicates) Pre-treatment	 Post-treatment
1	BASELINE (without suspended solids)	~8	-	265 (260,270)
2	without organic bases	11	265 (200,330)	265 (240,290)
4	without oxidizable & purgeable compounds	~8	290 (260,320)	290 (290,290)
5	without purgeable compounds	~8	290 (260,320)	285 (300,270)

Table 2: Results for the manipulations of the Toxicity Identification Evaluation (TIE) that produced a reduction of the acute toxicity of surface tailings pond water assessed using the Microtox assay. A change in the acute toxicity, relative to the IC_{50} at the initial sample pH (~8), with pH ranging from 6 to 8.5 (TIE manipulation # 6) would indicate that compounds whose relative ionization changes within that pH range contribute to the acute toxicity of surface tailings pond water.

#	TIE manipulations	pH	IC_{50} (mL/L) $\bar{x}$ (replicates) Pre-treatment	 Post-treatment	% Change or (↓ or ↑)
1	BASELINE (without suspended solids)	~8	-	265 (260,270)	-
2	without organic acids	2.5	205 (190,220)	>1000 (>1000,>1000)	↓ 100
3	without non-polar organic compounds	2.5	205 (190,220)	>1000 (>1000,>1000)	↓ 100
		~8	265 (260,270)	>1000 (>1000,>1000)	↓ 100
		11	265 (240,290)	>1000 (>1000,>1000)	↓ 100
6	graduated pH tests	6	-	250 (250)	↑
		7	-	310 (310)	↑
		~8	-	380 (380)	0
		8.5	-	380 (380)	0

Table 3: The change in acute toxicity as measured by Microtox for surface tailings water subjected to two manipulations of the Toxicity Identification Evaluation (TIE). An increase and subsequent decrease in the IT_{50} with increasing sodium thiosulphate or EDTA concentrations would implicate reducible compounds or cationic metals respectively as contributing to the acute toxicity of surface tailings pond water.

#	TIE manipulations	Dose (g/L) of $Na_2S_2O_3$ or EDTA added to 450 µL surface water/mL test sample	Post-treatment IT_{50} (min.) $\bar{x}$ (replicates)	Relative change in toxicity	Expected trend if a positive result
7	varying degrees of reducible compounds (treatment with $Na_2S_2O_3$)	0.0	11.8 (11.3,12.4)	-	-
		0.53	12.4 (10.5,14.2)	0	↓
		1.06	9.4 (8.8,10.0)	↑	↓
		1.58	7.8 (7.7,7.9)	↑	↑
8	varying degrees of complexed (less toxic) cationic metals (treatment with EDTA)	0.0	10.7 (12.8,8.7)	-	-
		0.04	10.1 (12.2,8.0)	0	↓
		0.08	7.6 (7.2,7.9)	↑	↓
		0.16	8.0 (7.3,8.7)	0	↓
		0.32	8.5 (9.0,8.1)	0	↑
		0.64	8.4 (8.3,8.5)	↑	↑↑

Table 4: Acute toxicity of fine tails interstitial water assessed using the Microtox assay for six of the manipulations of the Toxicity Identification Evaluation (TIE). A ↓ indicates a decrease in acute toxicity with treatment.

			IC_{50} (mL/L) $\bar{x}$ (replicates)		% Change
#	TIE manipulations	pH	Pre-treatment	Post-treatment	
1	BASELINE (without suspended solids)	~8	–	290 (300,280)	–
2	without organic acids	2.5	265 (250,280)	>1000 (>1000,>1000)	↓ 100
3	without nonpolar organic compounds	~8	290 (300,280)	>1000 (>1000,>1000)	↓ 100
4	without oxidizable & purgeable compounds	~8	290 (300,280)	340 (440,240)	↓ 10
5	without purgeable compounds	~8	290 (300,280)	360 (420,300)	↓ 15

Table 5: Acute toxicity as measured with *Daphnia magna* and rainbow trout for surface tailings pond water subjected to three of the TIE manipulations. A dash (–) indicates tests that were not performed on these samples. A change in the acute toxicity, with pH ranging from 6.5 to 8.5 (TIE manipulation # 6) would indicate that compounds whose relative ionization changes within this pH range contribute to the acute toxicity of surface tailings pond water.

			Daphnia magna			Rainbow trout		
			LC_{50} (mL/L) $\bar{x}$ (replicates)		% Change	LC_{50} (mL/L) or LT_{50} (hrs) $\bar{x}$ (replicates)		% Change or relative change
#	TIE manipulations	pH	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
1	BASELINE (without suspended solids)	~8	–	760 (800,710,770)	–	–	125 (120,130)	–
2	without organic acids	2.5	800 (800)	>1000 (>1000,>1000,>1000)	↓ 100	110 (110)	>1000 (>1000,>1000)	↓ 100
3	without non-polar organic compounds	~8	760 (800,710,770)	>1000 (>1000,>1000,>1000)	↓ 100	–	–	–
6	graduated pH tests	6.5	–	–	–	2.0	2.0 hrs	↑
		7.5	–	–	–	7.2	7.2 hrs	0
		8.5	–	–	–	168	168 hrs	↓

Table 6: The comparative acute toxicity of surface tailings pond water to Microtox, *Daphnia magna* and rainbow trout.

	Microtox vs. *Daphnia magna*			Microtox vs Rainbow trout		
	IC_{50} or LC_{50} (mL/L) $\bar{x}$ (replicates)		Microtox / Daphnia	IC_{50} or LC_{50} (mL/L) $\bar{x}$ (replicates)		Microtox / Trout
Sample	Microtox	*Daphnia magna*		Microtox	Rainbow trout	
BASELINE (without suspended solids)	290 (270,250,345)	760 (800,710,770)	0.38	355 (355)	125 (120,130)	2.8

ALUMINUM COMPLEXATION BY A NATURALLY OCCURRING FULVIC ACID: DETERMINATION OF CONDITIONAL STABILITY CONSTANTS AND PREDICTION OF INORGANIC AL LEVELS IN SYNTHETIC ACIDIC SOFT WATERS.

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ABSTRACT

During snowmelt, rivers on the Canadian Shield are subjected to a pulse of acidic water, including increased levels of Al and dissolved organic carbon (DOC). A significant proportion of this Al is complexed with DOC and these complexes do not appear to be implicated in the toxicity of Al observed with fish. In the context of toxicity bioassays, titration studies were conducted to determine the speciation of Al in acidic, organic, soft waters, representative of those on the Canadian Shield. Batch titration studies were done with a natural fulvic acid (FA) and a reconstituted soft water, at 10 °C. Studies involved three fulvic acid concentrations at pH 5.0 (5, 10 and 20 mg•L^{-1}) and one concentration, 10 mg•L^{-1}, at pH 4.5. Total [Al] ranged from 0 - 16.7 µM. Measurements of Al speciation used an automated colorimetric method to discriminate among total (acid reactive) Al, organic monomeric Al and inorganic monomeric Al. Titration data were plotted as Langmuir isotherms and conditional stability constants (log K_c) and complexation capacities (CC) were determined. The CC and log K_c values of the various curves were statistically similar, with the exception of the K_c value obtained for 5 mg•L^{-1} FA at pH 5.0. The experimentally determined CC and log K_c parameters and the chemical equilibrium modelling program MINEQL$^+$ were used to predict inorganic Al concentrations at various fulvic acid concentrations in fish bioassays. There was relatively good agreement between the predicted and measured inorganic [Al]. The use of experimental titration data coupled with MINEQL$^+$ equilibrium modelling allows for greater control of Al complexation in the bioassay exposure solutions.

INTRODUCTION

During periods of snowmelt, rivers on the Canadian Shield are subjected to a pulse of acidic water. Such acidic episodes have been monitored in rivers on Quebec's North Shore, such as the de la Trinité River; during spring runoff, the pH decreases while dissolved Al and dissolved organic carbon (DOC) levels increase (Fig. 1). These rivers are an important habitat for Atlantic salmon (*Salmo salar*), in particular juvenile salmon at this time of year. In acidified environments, Al is known to cause toxicity to aquatic organisms (Leivestad *et al.*, 1987; Lacroix, 1989; Potts and McWilliams, 1987) and may pose a threat to these salmon populations.

Fulvic and humic acids account for approximately 50% of the DOC in these North Shore rivers, and a high proportion of the Al is present as dissolved organic complexes (Hansen and Campbell, 1987 Campbell *et al.*, 1992). Laboratory studies suggest that similar Al-organic complexes are not implicated in the toxicity of Al observed with fish (Lydersen et *al.*, 1990; Parkhurst *et al.*, 1990). As part of a study examining the toxicity of Al to Atlantic salmon, bioassays involving exposure of juvenile fish to Al-organic solutions were contemplated. The experimental design required that we be able to predict the concentrations of inorganic Al in acidic waters containing different concentrations of DOC.

Batch titration studies were conducted to determine the complexation behaviour of Al in acidic, organic, reconstituted soft waters, representative of those present on the Canadian Shield during snowmelt. The results were used in the chemical equilibrium program MINEQL^{+} (Schecher and McAvoy, 1991) to calculate the speciation of Al at various concentrations of fulvic acid and total Al.

MATERIALS AND METHODS

Titration studies were done with a soil fulvic acid (FA) that is available commercially (Ecolinc, Inc., Roxboro, Quebec) and has been well characterized (Langford *et al.*, 1983). All experiments were conducted using a reconstituted soft water, prepared by the addition of the following salts ($mg \bullet L^{-1}$; M) to deionized water: $NaHCO_3$ (12; 143); $CaSO_4 \bullet 2H_2O$ (7.5; 44); $MgSO_4$ (7.5; 62); KCl (0.5; 7).

Batch titrations

Batch titrations were performed at three concentrations of fulvic acid (FA) at pH 5.0 (5, 10 and 20 $mg \bullet L^{-1}$) and one [FA] (10 $mg \bullet L^{-1}$) at pH 4.5. Each titration series consisted of 21 incremental additions of Al and a control; each series was analyzed three times. For each [FA], an appropriate 2 L test solution was prepared by dilution of a concentrated stock solution in reconstituted soft water. The [FA] in the test solution was verified by absorbance at 400 nm, in comparison with a standard curve. The test solution was then apportioned into 22 containers, with 80 mL of solution in each. Amounts of a concentrated Al solution, ranging from 0 to 4 mL, were added; as necessary, reconstituted soft water was also added to complete the volume to 84 mL. The pH was adjusted to pH 5.0 or pH 4.5 and the solutions were equilibrated at 10 °C for a minimum of 96 h prior to analysis. Final [Al] ranged from 0 - 9.4 µM at 5 $mg \bullet L^{-1}$ FA, 0 - 14.1 µM at 10 $mg \bullet L^{-1}$ and 0 - 17.8 µM at 20 $mg \bullet L^{-1}$ FA .

Al analysis

Al was measured with an automated spectrophotometric technique (Rogeberg and Henriksen, 1985) based upon the reaction of monomeric Al with pyrocatechol violet (PCV). This method allows the differentiation of total monomeric Al (Al_{cm}) and organic monomeric Al (Al_o); inorganic monomeric Al (Al_i) is then estimated by the difference $Al_{cm} - Al_o$. A modification of the technique for our laboratory involved the determination of total, acid-reactive Al (Al_t). This involved the acidification (to pH 1.0 with concentrated trace metal grade HCl) of a 10 mL subsample, which was then left at room temperature for 48 h before analysis. Total acid-reactive Al measurements averaged 85% of nominal concentrations (range 80 - 96%).

Dialysis

Dialysis bags (Spectra-Por 7, 1000 MWCO, for trace metal analysis) were rinsed in Millipore deionized water prior to use, filled with 10 mL of inorganic synthetic soft water and clamped shut. They were then placed into the bioassay tanks (2 per basin) for a period of 96 h. Preliminary studies had shown that a period of 48 h was necessary to obtain an equilibrium for Al between the inside and outside of the bag. After 96 h, the bags were removed from the tanks, rinsed in deionized water and the contents were analyzed for inorganic Al as described above.

Titration analysis

Total Al concentrations were initially plotted against bound Al in order to determine the degree of saturation of the ligand. Only those points falling on the rising limb, or the non-saturated portion of the curve, were used for plotting the isotherm curves. Langmuir isotherms were then determined by linear regression of Al_{free}/Al_{bound} versus Alfree (Pott *et al.*, 1985), according to the equation:

Bound Al was calculated as Al_t - inorganic (free) Al. The complexation capacities (CC) and conditional stability constants (K_c) were determined from the isotherms as follows:

The CC values were normalized with respect to the fulvic acid concentration. The CC and K_c values were compared statistically using an analysis of variance with a significance level of 0.05. Significant differences among means were detected using the Student-Newman-Keuls *a posteri* procedure.

Al speciation: determined from the Langmuir isotherms

The Langmuir equation, with the experimentally determined CC and K_c values, was used to calculate inorganic [Al] at various concentrations of total Al. The Langmuir relationship was transformed into a quadratic equation (eq^n 4) and solved for "R", and thus for Al_{free}:

where: $R = Al_f / Al_t$; $K' = 1 / CC{\bullet}K_c$; and $C' = Al_t / CC$.

Al speciation: MINEQL$^+$ calculations

Complexation constants used in chemical equilibrium models, such as MINEQL$^+$, are derived from reactions involving the free metal ion. Our experimentally determined constants were determined from measurements of total inorganic Al and thus had to be adjusted to account for the different proportions of Al^{3+} at our two pH values. We obtained estimates of the proportions of Al^{3+} present at pH 4.5 and pH 5.0 by running MINEQL$^+$ in an inorganic matrix - in the absence of FA, with a range of [Al] from 0.37 to 9.3 μM. At pH 4.5, Al [illegible]s the predominant inorganic species (66%), while at pH 5.0, this proportion decreases to approximately 20%. The best Langmuir curve at each experimental condition was then selected and the free Al measurements were converted into estimates of [Al^{3+}]. The linear regressions were recalculated and "corrected" experimental log K_c values were obtained (Table 2).

The corrected experimental complexation constants were then entered into the chemical equilibrium program MINEQL$^+$ to calculate the speciation of Al at different concentrations of FA. The program was run allowing for the precipitation of $Al(OH)_3(s)$ with micro-crystalline gibbsite as the controlling phase. Equilibrium constants (log K) for the inorganic complexes of Al were taken from Schecher and Driscoll (1987): $AlOH^{2+}$: - 4.97; $Al(OH)_2^+$: - 8.67, $Al(OH)_4^-$: - 23.30; $Al(OH)_3$ aq: -15.00; $AlSO_4^+$: 3.02; $Al(SO_4)_2^-$: 4.92; $Al(OH)_3$ (s): -8.35. The constants were corrected for a temperature of 10 °C.

RESULTS AND DISCUSSION

Langmuir curves

Linear regression values (r^2) of the Langmuir curves (Fig. 2) ranged from 0.99 to 0.88 and there was close agreement in intercept and slope values among the replicates. Curves at FA concentrations of 5 and 10

$mg \bullet L^{-1}$ were fairly linear, suggesting that the Al-FA complex could be modeled as 1:1 binding. At 20 $mg \bullet L^{-1}$ FA, a moderately improved fit was obtained by plotting the data with a polynomial function. However, a single site model also adequately described these data. The accuracy of the fit was not notably improved using Scatchard plots.

The calculated log K_c and CC values at different [FA] are shown in Table 1. The CC values were all in the same range and there were no significant differences at different [FA], nor at the same [FA] at pH 4.5 and pH 5.0. In addition, with one exception, all of the conditional stability constants were not significantly different. The log K_c at the [FA] of 5 $mg \bullet L^{-1}$ is slightly higher than the other values, and the K_c value is significantly different than the other values ($p > 0.01$). This difference may be related to the interference in the colorimetric technique caused by the presence of organic matter (Royset and Sullivan, 1986). At low FA, this interference was negligible, allowing for accurate measurement of low concentrations of inorganic Al. At 10 and 20 $mg \bullet L^{-1}$ FA, this interference made determination of free Al difficult at low concentrations and could have influenced the accuracy of our titration curves at [FA] > 5 $mg \bullet L^{-1}$. However, our log K_c at 5 $mg \bullet L^{-1}$ also differs from values reported in the literature (see below), and there is no obvious explanation for these differences.

With the exception of the pH 5.0 - 5 $mg \bullet L^{-1}$ FA titration, our log K_c values are in agreement with estimates for Al-FA complexes at pH 5.0 (log K_c 5.5 - 6.0) reported in other studies (Potts *et al.*, 1985; Campbell *et al.*, 1992). Campbell *et al.* (1992) performed similar batch titrations with DOM from Quebec North Shore salmon rivers. They obtained log K_c values ranging from 6.17 - 6.23, and CC values ranging from 0.72 to 1.10, i.e. comparable to those found in this study, with the exception noted above. In this study, the affinity for Al, as represented by the conditional constants, is similar at the two pH values we tested (pH 5.0 and pH 4.5; Table 1). A lower log K_c might have been expected at the lower pH as more FA sites become protonated, thereby reducing complexation. However, at lower pH the $[Al^{3+}]$ also increases, which would favour complexation - the conditional constant reflects both of these competing processes (Campbell *et al.*, 1992).

The "corrected" log K_c values (expressed in terms of Al^{3+} rather than Al_i ; Table 2) are greater than their corresponding values in Table 1. These higher log K_c values take into account the proportion of free ion at the two experimental pH values (66 % at pH 4.5, 20 % at pH 5.0). The CC values (not shown) are identical to those of the original curves, as no correction was employed for bound Al. We used the average CC values displayed in Table 1 in later calculations with the "corrected" log K_c values.

Al speciation calculations

We verified these "corrected" constants by using them to calculate Al speciation in the presence of fulvic acid, and then comparing these results with those obtained directly from the Langmuir equation. First, for a given [FA], the experimentally determined constants in Table 1 were entered into the Langmuir equation and inorganic Al concentrations were calculated over a range of total Al from 0.37 - 7.4 μM ("equation" values in Tables 3 and 4). We compared these calculated values with the levels of Al_i actually measured at the same $[Al_t]$ in the titration series and obtained similar values (data not shown). In addition, we entered the "corrected" constants in Table 2 into MINEQL$^+$ and calculated $[Al_i]$ over the same range of Al_t, also shown in Tables 3 and 4. There is excellent agreement (all $r^2 > 0.99$) between the two methods for predicting inorganic Al at pH 4.5 (10 mg•L^{-1} FA, Table 3) and at pH 5.0 (5, 10 and 20 mg•L^{-1} FA, Table 4).

MINEQL^{+} calculations:the effects of pH and [FA] on the binding of Al by FA

When the MINEQL^{+} calculations in Tables 3 and 4 are compared for the same [Al_t] and [FA] , it is possible to judge the effects of pH on the complexation of Al. For example, for [Al_t] = 7.4 μM and at a [FA] of 10 mg•L^{-1}, the concentrations of inorganic Al are identical at pH 4.5 and pH 5.0 (1.8 μM). This difference in pH is evidently not sufficient to favour either of the competing processes mentioned previously (greater competition from increased [H^{+}], increased complexation with increased [Al^{3+}]).

Conditional constants determined at one ligand concentration are often used for the prediction of metal binding at other ligand concentrations. We attempted to evaluate this practice for Al and FA at a total Al concentration of 7.4 μM, by using the pH 5.0 - 10 mg•L^{-1} conditional constant (log K_c = 6.54, Table 2) to calculate the concentration of bound Al at 5 and 20 mg•L^{-1} FA. In Figure 3, these values are compared with the bound Al concentration calculated using the "specific" log K_c determined at these [FA] (log 7.27 at 5 mg•L^{-1} FA; log 6.27 at 20 mg•L^{-1} FA, Table 2), at the same [Al_t] of 7.4 μM. At a [FA] of 20 mg•L^{-1}, both the "specific" and the pH 5.0 - 10 mg•L^{-1} conditional constant result in similar values for bound Al (6.1 μM and 6.7 μM). In contrast, at a [FA] of 5 mg•L^{-1} , the predicted amount of bound Al is only 3.7 μM using the pH 5.0 - 10 mg•L^{-1} conditional constant, as opposed to 5.2 μM obtained with the "specific" log K_c of 6.27. Given the known influence of Al:fulvic acid ratios of the strength of the Al-FA binding (e.g., Browne and Driscoll, 1993), it is clearly preferable not to use complexation constants to calculate Al speciation outside the range of conditions in which the constants were originally determined.

Inorganic Al in bioassays

Finally, the MINEQL^{+} program with the corrected constants was used to determine the amount of Al to add to obtain constant [Al_i] at different [FA] in a bioassay with fish. This assay, at pH 5.0, involved varying [FA] while attempting to maintain [Al_i] constant (Fig.4). Samples were taken directly from the bioassay tanks and analyzed colorimetrically as described in the methods (Al_m - Al_o). The dialysis bag samples were also analyzed colorimetrically for inorganic (dialysable) Al at the end of the assay. There was relatively good agreement between the two sampling techniques used to determine inorganic Al experimentally. However, the predictions derived from the MINEQL^{+} calculations overestimated Al_i. These were static bioassays, without renewal of the solutions, and it is possible that losses of inorganic Al occurred, due to sorption to the containers and organisms. However, the MINEQL^{+} calculations did predict nearly equivalent concentrations of Al_i at different [Al_t], as observed experimentally, the critical factor in this experimental design.

CONCLUSIONS

In conclusion, we found the use of titration data coupled with MINEQL^{+} equilibrium modelling to be useful for the prediction of inorganic Al concentrations in acidic organic soft waters. The ability to compare expected and observed concentrations of Al allows for greater control of the complex changes in solution chemistry which may occur during bioassays. However, the use of models such as MINEQL$^+$ should be done with caution. We suggest that the results of chemical equilibrium modelling be compared with titration data to verify the accuracy of the predictions. We also stress the importance of using free-ion concentrations for the calculation of conditional constants derived from titration data. This is particularly important for metals which undergo considerable changes in inorganic speciation with pH, and for which no reliable methods exists for direct measurement of the free-ion concentration. The use of actual titration data specific to our fulvic acid, as opposed to literature values, also contributed to the relatively good agreement we observed between measured and predicted inorganic Al.

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Figure 1. Chemistry of the de la Trinité River during snowmelt.

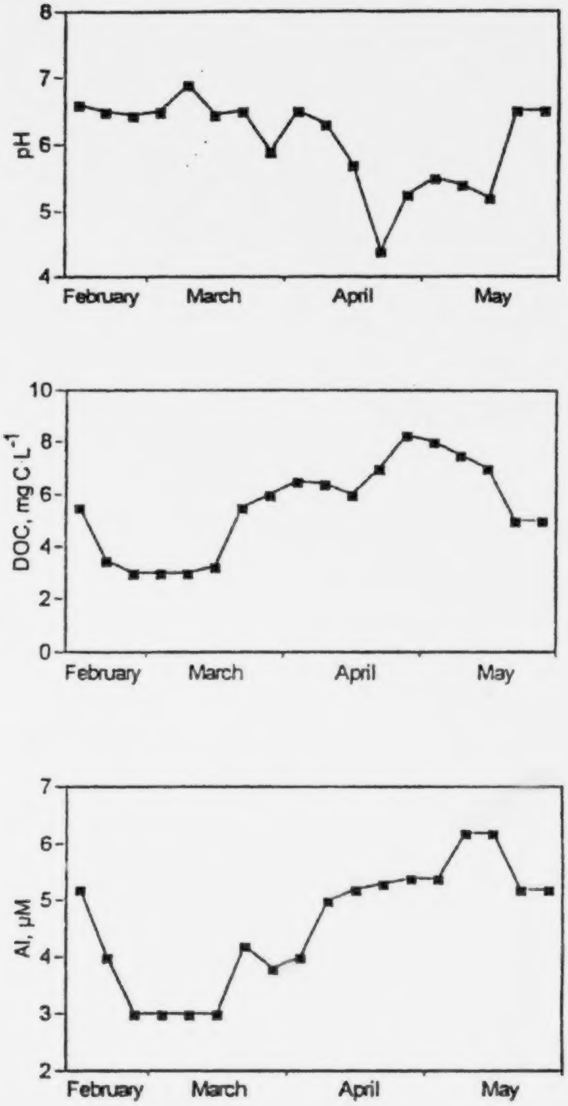
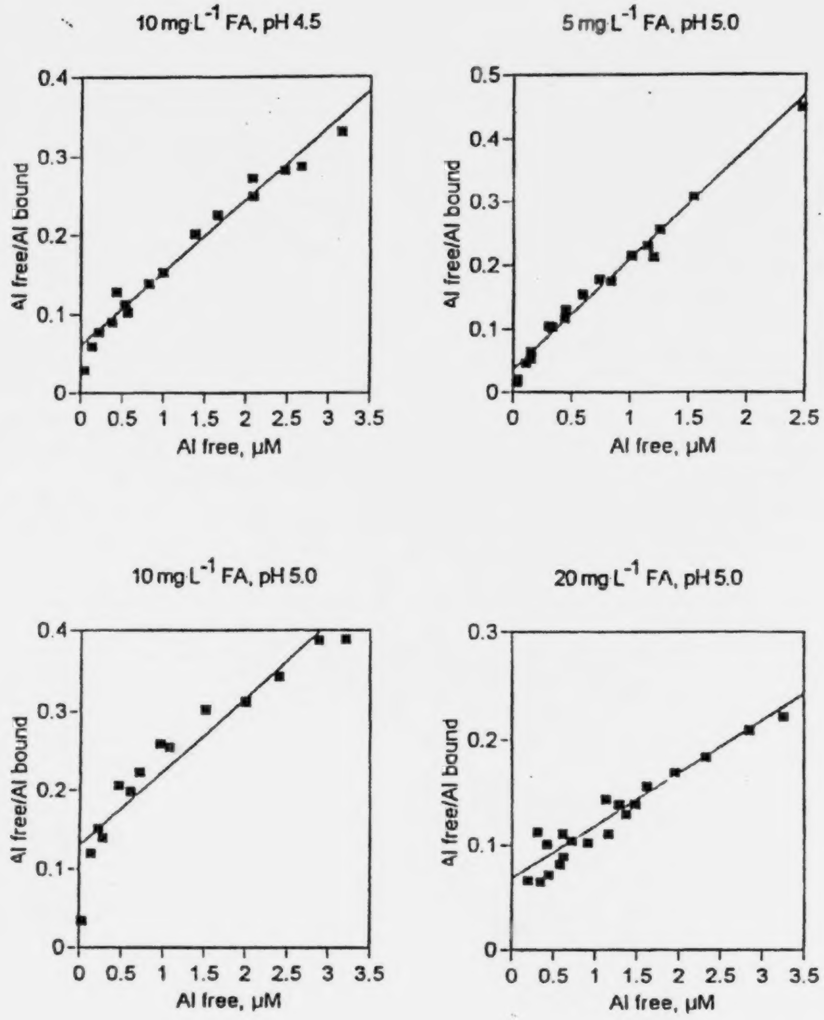


Figure 2. Langmuir isotherms determined from titration data with fulvic acid (FA).



10 mg·L^{-1} FA, pH 4.5

5 mg·L^{-1} FA, pH 5.0

10 mg·L^{-1} FA, pH 5.0

20 mg·L^{-1} FA, pH 5.0

Figure 3. Bound Al calculated using MINEQL$^+$ for three [FA] at pH 5.0, at a total Al of 7.4 μM. Bound Al was calculated with the log K_a specific for each [FA] (■, left hand bar), and with the log K_a for 10 mg·L^{-1} FA (= 6.54) to estimate bound Al at 5 and 20 mg·L^{-1} FA (□, right hand bar).

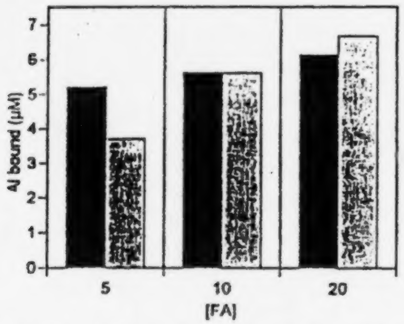


Figure. 4. Inorganic Al concentrations (μM) in bioassay tanks at pH 5.0. [Al$_i$] was measured in samples taken directly from the tanks ("colorimetry", mean ± S.D.) and on the contents of dialysis bags ("dialysis"). Predicted [Al$_i$] ("MINEQL") were calculated using MINEQL$^+$ and the constants in Tables 1 and 2.

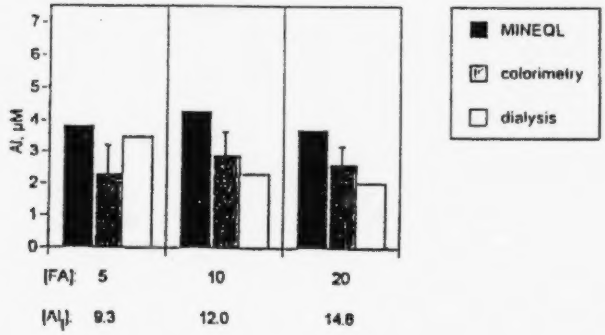


Table 1. Means (S.D.) of log K_a and CC values determined from Langmuir plots of Al-FA titration data.

[FA] , mg·L^{-1}	pH	range of [Al], μM	log K_a	CC (μmoles Al · mg^{-1} FA)
5	5.0	0.18 - 9.3	6.60 (0.08)	1.19 (0.10)
10	5.0	0.37 - 13.3	6.08 (0.18)	0.95 (0.16)
20	5.0	0.37 - 17.8	5.85 (0.18)	1.00 (0.08)
10	4.5	0.18 - 14.1	6.16 (0.19)	1.08 (0.12)

Table 2. Recalculated log K_a values obtained using estimates of [Al^{3+}] for Al_{free}.

pH, [FA]	log K_a
pH 5.0, 5 mg·L^{-1}	7.37
pH 5.0, 10 mg·L^{-1}	6.54
pH 5.0, 20 mg·L^{-1}	6.27
pH 4.5, 10 mg·L^{-1}	6.18

Table 3. Inorganic Al concentrations at pH 4.5 and a [FA] of 10 mg· L^{-1}. Values for $[Al_i]$ were either calculated directly from the Langmuir equation ("equation") or obtained by using the MINEQL$^+$ chemical equilibrium program with the constants from Table1 (CC values) and Table 2 ("corrected" Log K_a values).

Al_t		Al_i (μM)	Al_i (μM)
(mg·L^{-1})	(μM)	MINEQL$^+$	equation
10	0.37	0.04	0.04
20	0.74	0.09	0.08
50	1.85	0.24	0.22
70	2.60	0.36	0.33
100	3.70	0.59	0.53
120	4.44	0.76	0.70
150	5.55	1.09	1.00
200	7.41	1.81	1.68
250	9.26	2.79	2.64

Table 4 . Inorganic Al concentrations at pH 5.0 and [FA] concentrations of 5, 10 and 20 mg·L^{-1}. Values of [Al$_i$] (μM) were either calculated directly from the Langmuir equation ("equation") or obtained by using the MINEQL$^+$ chemical equilibrium program with the constants from Table1 (CC values) and Table 2 ("corrected" Log K$_a$ values).

Al$_i$		5 mg·L^{-1}		10 mg·L^{-1}		20 mg·L^{-1}	
mg·L^{-1}	μM	MINEQL$^+$	equation	MINEQL$^+$	equation	MINEQL$^+$	equation
10	0.37	0.02	0.01	0.05	0.04	0.05	0.04
20	0.74	0.03	0.03	0.10	0.09	0.10	0.09
50	1.85	0.10	0.09	0.28	0.25	0.25	0.23
70	2.59	0.16	0.15	0.41	0.37	0.36	0.33
100	3.70	0.32	0.30	0.64	0.57	0.54	0.49
120	4.44	0.50	0.46	0.82	0.74	0.67	0.61
150	5.56	0.93	0.88	1.13	1.02	0.89	0.80
200	7.41	2.19	2.14	1.79	1.63	1.27	1.16
250	9.26	3.81	3.78	2.63	2.43	1.75	1.59

RÉSUMÉS/ ABSTRACTS

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ÉCOLOGIE DES GRANDS FLEUVES

Un fleuve est une masse d'eau en mouvement : il a une puissance proportionnelle au carré de sa vitesse qui est fonction de la pente. La puissance de l'eau est utilisée pour vaincre les frottements (rugosité du lit, etc.) et transporter les matériaux arrachés au lit de la rivière. Les eaux courantes constituent ainsi un vaste système d'érosion et de redistribution des matériaux terrestres et ont un profil en long déterminé. Le long de celui-ci, des facteurs varient régulièrement de l'amont à l'aval : réduction de la pente et de la vitesse, sédiments de plus en plus fins, température et teneur de l'eau en sels dissous de plus en plus élevées. On observe, de l'amont vers l'aval, un continuum des facteurs écologiques. Au concept de continuum paraît s'opposer celui des zonations, qui implique des discontinuités. En fait, ce sont deux concepts complémentaires, que permet de comprendre la théorie des catastrophes. En effet, l'autonomie d'une espèce vis-à-vis d'un facteur écologique s'exerce entre deux limites. A la variation continue de ce facteur répond la discontinuité (le point de catastrophe) dans la répartition des espèces. Cette discontinuité est évidente lorsqu'on étudie la succession amont aval des espèces d'un même genre ou de genres voisins; elle devient moins nette lorsqu'on envisage le peuplement amont-aval dans son ensemble. Les possibilités d'aménagements seront différentes suivant le niveau auquel elles seront réalisées sur un profil d'équilibre et les conséquences en seront également différentes. Un déversement polluant domestique ou industriel a ainsi des effets distincts. Sur le cours supérieur, il faut connaître l'effet destructeur immédiat sur le peuplement. Sur le cours inférieur, il faut étudier l'incorporation des éléments rejetés dans l'écosystème, leur transformation dans le temps et leur élimination en fonction de la durée de rétention de l'eau.

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COMPARISON OF HEAVY METAL CONTENTS BETWEEN 1989-1991 AND 1993 IN SMELT AND IN SELECTED BY-CATCH SPECIES COLLECTED FROM THE MIRAMICHI ESTUARY

The objective of this study was to compare Hg and heavy metal concentrations in biota collected from the Miramichi estuary during 1993 with those collected during the MREAC study conducted between 1989 and 1991. Samples were collected as bycatch from the winter smelt fishery in January and February 1993, from two sites in the Miramichi estuary. In addition to smelt (*Osmerus mordax*); tomcod (*Microgadus tomcod*), winter flounder (*Pseudopleuronectes americanus*), smooth flounder (*Liopsetta putnami*) and crangon (*Crangon septemspinosa*) were collected for this study. All biota were analyzed for mercury content, and smelts were further analyzed for Cu, Cd, Pb and Zn. All concentrations from 1993 were similar to those reported in the MREAC study with the exception of Hg in tomcod livers, which was 10 fold higher in the MREAC study (0.40 µg/g, dry weight) than concentrations measured in 1993 (0.05 µg/g, dry weight). This study contributes to an overall assessment of contaminants in estuarine biota of the Gulf coasts of N.B. and N.S. undertaken as part of the Green Plan Toxic Chemicals Program.

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BALCH[1] G.C., GOUDEY[1] J.S., METCALFE[2] C.D.

A JAPANESE MEDAKA EGG EXPOSURE PROTOCOL FOR ASSESSING THE PRESENCE OF SEDIMENT ASSOCIATED EMBRYO TOXICANTS

Contaminated sediments collected from Hamilton Harbour, Ontario, were tested for the presence of compounds toxic to developing embryos of Japanese medaka. One egg ($<$8 h post fertilization) was placed into a 4 mL glass vial with either sediment, pore water, or an organic extract of the sediment. Each vial was capped and incubated in the dark at 25 ± 2° C till hatch of control fish. Direct sediment exposure was investigated using a serial dilution series prepared by placing 0.5 grams of a sediment/silica sand mixture into a 4 mL glass vial with 1 mL of reconstituted waters. Pore water was tested in an identical manner, except pore water replaced the reconstituted water and no sediment was used. Organic extracts were prepared, and fractionated using column chromatography. Microlitre quantities of extract or subfraction were evaporated in vials and rehydrated with 1 mL of reconstituted water. Sediment quantities $\geq$ 1.8 mg (dry wt.) per vial caused near 100 % cumulative embryo death by day 5. Equivalent amounts of a "clean" reference sediment did not cause death by day 20. A dose response relationship for embryo mortality was observed for sediment weights $<$ 1.8 mg/vial. Embryos exposed to undiluted pore water showed delayed development and embryonic lesions which were not evident in diluted samples. The unfractionated sediment extract and the PAH containing fraction were found to be the most embryotoxic.

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BARTELL[1] S.M.

CONCEPTS AND METHODS FOR ASSESSING ECOLOGICAL RISKS IN A WATERSHED CONTEXT

Ecological risk assessments are inherently spatial: ecological resources potentially at risk operate at or occupy characteristic spatial scales. A watershed represents one convenient and important spatial scale for risk assessment. Hydrologic processes that define watersheds also play a determining role in the transport and distribution of many chemical contaminants in nature. Water movement is also important in influencing the flow of energy and the cycling of materials that are essential for sustaining the watershed and its inhabitants. With water as a defining medium, watersheds are conveniently bounded for risk assessment, from vantage points in both basic ecology and environmental chemistry. This presentation will discuss concepts and methods for delineating responses to disturbance, quantifying exposure, forecasting effects, and characterizing ecological risks for watersheds. Concepts borrowed from basic disturbance theory and landscape ecology will be used to construct a framework for ecological risk estimation applicable to watersheds. Critical issues regarding essential data, spatial statistics, dynamic models of chemical fate and ecological effects, geographical information systems, and sources of uncertainty will be discussed.

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BERMINGHAM[1] N, BLAISE[1] C., BOUDREAULT[1] D.

BAREME D'ÉVALUATION DE L'ÉCOTOXICITÉ POTENTIELLE DES EFFLUENTS INDUSTRIELS

Un indice a été développé pour évaluer et comparer le potentiel toxique des effluents industriels. Il intègre les résultats de différents tests de toxicité aquatique (Microtox®, inhibition de croissance algale de *Selenastrum capricornutum*, létalité et inhibition de la reproduction de crustacé *Ceriodaphnia dubia*, génotoxicité avec le SOS Chromotest), la persistance de la toxicité (reprise des tests avec l'effluent soumis à une biodégradation durant 5 jours) et le débit de l'effluent. L'ensemble est exprimé par une valeur sur une échelle logarithmique variant de 0 à 10. La structure mathématique de l'indice est suffisamment flexible pour permettre l'ajout ou le retrait éventuel de certains tests. L'évaluation et la comparaison du potentiel toxique des effluents repose sur un ensemble de bioessais utilisant divers organismes représentatifs de plusieurs niveaux trophiques (décomposeurs, producteurs primaires, consommateurs) et permettant d'analyser plusieurs niveaux et types de toxicité (létalité aiguë et chronique, sublétalité aiguë et chronique). Cette stratégie couplée à une détermination de la persistance de la toxicité (représentative du devenir de la toxicité dans l'environnement) ainsi qu'à la mesure du débit de l'effluent (permettant de raisonner en termes de charge toxique), constitue une tentative pour réunir divers concepts écotoxicologiques dans un outil de travail simple et utile. Le mode d'expression des résultats sous forme d'une unique valeur facilite la diffusion auprès du public habitué à ce type d'information synthétique (par exemple, échelle de Richter, échelle de pH). Le modèle peut servir à la gestion des effluents à l'intérieur d'une usine, au contrôle temporel de la toxicité des effluents ou à l'évaluation du succès des mesures de détoxification.

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BIRKHOLZ[1] D.A., GOUDEY[2] S.

ISOLATION AND IDENTIFICATION OF TOXICANTS PRESENT IN CREOSOTE CONTAMINATED GROUNDWATER

A sample of groundwater, taken in the vicinity of a creosote-contaminated site, was subjected to the Toxicity Identification Evaluation process. Toxicants were isolated and identified by applying the pH adjustment/solid phase extraction test. Solid phase extraction at pH ambient, pH 3.0 and pH 9.0 revealed total reduction in toxicity. Toxicants were recovered from the solid phase extraction cartridges by stripping with 50 % methanol : 50 % water and 75 % methanol : 25 % water. All of the toxicants were accounted for in these two fractions, irrespective of extraction pH. This observation supported the presence of toxic amphoteric compounds, i.e. compounds having both basic and acidic properties. Analyses of the solid phase extracts [both 50 % and 75 % methanol fractions] were performed using liquid chromatography/mass spectrometry [LC/MS] and gas chromatography/mass spectrometry [GC/MS]. GC/MS analyses were performed after solvent exchange with chloroform and derivatization. The major components found using GC/MS in the 50 % methanol fraction were: hydroxylated polycyclic aromatic nitrogen heterocycles [HPANH], polycyclic aromatic hydrocarbons [PAH], chlorophenols [CP], hydroxylated polycyclic aromatic hydrocarbons [HPAH], phenols [P], and polycyclic aromatic nitrogen heterocycles [PANH]. Concentrations were estimated to be : HPANH > PAH > CP > HPAH > P > PANH. For the 75 % methanol fraction the major components found using GC/MS and estimated concentrations were : PAH > P > CP > HPAH > PANH > HPANH. Analysis of the 50 % and 75 % methanol fractions using LC/MS revealed the major components to be hydroxylated quinolines [i.e. HPANH]. The identification of HPANH [which are amphoteric] and HPAH suggests that these compounds represent microbrial transformation products of PANH and PAH which are present in creosote. The toxic nature of HPANH and HPAH should be taken into consideration during site remediation.

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BISAILLON[1] J.-G., BEAUDET[1] R., LÉPINE[1] F.

ÉTUDE MICROBIOLOGIQUE DE LA DÉGRADATION DE COMPOSÉS AROMATIQUES POLLUANTS

Les activités agricoles et industrielles introduisent une grande variété de composés aromatiques polluants dans l'environnement. L'étude des microorganismes impliqués dans la dégradation de ces composés aromatiques est importante car ils sont les ouvriers dans les procédés de traitement. Des travaux ont été réalisés sur différents types d'effluents soit agricole (lisier de porc), industriel (pâte et papier, raffinerie), municipal (lixiviat de site d'enfouissement) et domestique (fosse septique) et de sols contaminés (hydrocarbures). Trois exemples serviront à illustrer les travaux que nous effectuons. Pour le traitement d'effluents contaminés, des consortiums de bactéries anaérobies ont été sélectionnés pour leur capacité à dégrader soit le phénol, soit le pentachlorophénol par fermentation méthanique. Le phénol est transformé en acide benzoïque par des bactéries sporulées appartenant au genre *Clostridium*. A l'aide de réacteurs anaérobies à film fixe, il a été démontré que le phénol contenu dans un effluent pétrochimique et le pentachlorophénol contenu dans un extrait de résidu de bois contaminé pouvaient être dégradés efficacement. De plus, la toxicité de cet effluent et de cet extrait a été réduite suite au traitement. Au niveau des sols, la flore indigène aérobie de la sablière Thouin s'est avérée capable de dégrader les hydrocarbures aromatiques polycycliques (HAP) contaminants ce site. La majorité des souches qui ont été isolées pour leur capacité à croître en présence d'hydrocarbures comme principale source de carbone appartiennent au *Pseudomonas* du groupe *fluorescens*. Suite au traitement du sol en réacteur par la flore indigène aérobie, la toxicité du sol a été réduite par un facteur de trois. Une meilleure compréhension des différents aspects microbiologiques impliqués dans les procédés de traitement devrait permettre d'optimiser leur performance. Ainsi, les traitements proposés ne seront pas empiriques mais basés sur des connaissances scientifiques solides.

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BLAISE[1] C.

APPLICATIONS PRATIQUES AVEC MICROALGUES POUR L'ÉVALUATION DES DANGERS DE CONTAMINANTS RELARGUÉS EN MILIEU AQUATIQUE

En écotoxicologie aquatique, l'évaluation multitrophique s'avère péremptoire pour porter un diagnostic fiable envers le stress chimique. Les microalgues, en particulier, peuvent être employées profitablement pour l'entreprise d'études innovatrices et polyvalentes de xénobiotiques et de rejets liquides complexes, avec des procédures rentables. Depuis une décennie, divers produits chimiques (métaux, organiques variés, herbicides) et effluents industriels ont fait l'objet d'études de phytotoxicité avec l'aide de l'algue verte *Selenastrum capricornutum* dans nos laboratoires. Au fil des ans, diverses techniques bioanalytiques se sont greffées à nos investigations phytotoxicologiques. Ainsi, le couplage (1) de procédures miniaturisées, (2) de mesures d'effets pertinents (effets algistatiques/algicidaux, effetsd'exposition/récupération), (3) de bioindicateurs auxiliaires (Microtox®, SOS Chromotest), et (4) de nouvelles technologies (cytométrie en flux, filtration par Multiscreen), aura contribué à l'amélioration de notre pouvoir de dépistage et de diagnostic écotoxicologiques. Un résumé des principales applications réalisées avec microalgues fera l'objet de cette présentation. Certaines perspectives de recherches à venir seront également évoquées.

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BOERSMA[1] D.

DATA QUALITY ISSUES FOR ECOLOGICAL ASSESSMENTS

Previous presentations in this session have discussed the difficulties encountered when completing ecological assessments that are lacking data. However, problems arose even when the required data were available. These data quality problems included the use of outdated or incorrect protocols, lack of reporting on details critical to the assessment of data quality, and lack of discussion on the data reported. The purpose of this presentation is to describe common data quality problems using examples from the 44 priority substance assessments, and to make suggestions as to how such problems can be minimized in the future.

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BONZONGO[1] J.C., PLANAS[1] D., PICHET[1] P., VAN TRA[1] H.

ACCUMULATION OF MERCURY AND METHYLMERCURY BY PERIPHYTON COMMUNITY

A complex matrix of biotic and abiotic materials accumulating on submerged natural and artificial substrates in littoral stations of a hydroelectric reservoir and a natural lake (in western Québec) were collected during the summer of 1992. Total mercury and methylmercury content in these samples was determined and correlated with the indicators of biotic components (Chla, microbial activity, bacterial number). Dry weight concentrations of mercury varied as follows : 100 to 200 ng/g in the natural lake and 200 to 1400 ng/g in the hydroelectric reservoir. Methylmercury represented less than 2 % of total mercury in the natural lake, but constituted around 20 % in the perturbed system. Partial correlation coefficients between the total mercury and methylmercury of periphyton and chlorophylla were not significant. However, methylmercury concentrations were strongly correlated with microbial activity, which was estimated by measuring electron transport system activity, and were also a power function of bacterial number. Our results indicated that periphyton, the lowest trophic level in these aquatic ecosystems, is a potential mercury accumulation layer, and can play a role in the release of methylmercury to the food chain in hydroelectric reservoirs.

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BOWES[1] M.J., TOBE[1] S.S., ZIMMERMAN[1] A.P.

INDUCTION OF A SMALL, METAL-BINDING PROTEIN (METALLOTHIONEIN) IN RESPONSE TO CADMIUM EXPOSURE IN GAMMARUS

Contamination of natural water systems with heavy metals has led to a need for the development of indicators of exposure. We are in the process of evaluating metallothionein (MT) induction in Gammarus as an indicator of cadmium exposure. Gammarus was selected as the test organism because it is readily available in urban river systems, which are more heavily impacted by human activity. With the use of radioimmunoassay, the induction of MT may be a useful indicator of metal exposure. Other workers have already had some success defining the relationship between dose and MT concentration. (Comp. Biochem. Physiol. 1989.93C:345-347). Gammarus was collected from selected urban rivers in the vicinity of Metropolitan Toronto, exposed to sublethal doses of cadmium, homogenized, and the supernatant subjected to a protocol based on the work of Roesijadi and Fowler. (Methods in Enzymology 1991.205:263-273). This protocol includes the crude separation of the protein by gel chromatography, identification of the Cd-MT- containing fraction by atomic absorption spectrophotometry, and further purification by high-performance liquid chromatography. Results relating body burden of cadmium with MT response will be discussed.

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BROCHU[1] C., MOORE[1] S., PELLETIER[2] E.

DISTRIBUTION DES DIOXINES ET FURANES POLYCHLORÉS DANS LES SÉDIMENTS ET CERTAINS ORGANISMES DU FJORD DU SAGUENAY

Dans le cadre d'un programme de caractérisation des contaminants organiques dans les sédiments et les organismes du Saguenay, plusieurs échantillons furent analysés afin d'évaluer la distribution des dioxines et furanes polychlorés dans cet environnement. Des sous-échantillons de deux carottes de sédiments prélevées dans le Saguenay, ainsi que de deux sites contrôles, une à la Baie des Milles Vaches et une dans la Baie des Anglais ont permis d'établir le profil de la contamination en dioxines et furanes en fonction du temps. Les résultats démontrent que depuis un certain nombre d'années les émissions en dioxines et furanes semblent diminuer. Les congénères prédominants dans le Saguenay et la Baie des Milles Vaches sont les octachlorodibenzo-*p*-dioxines et furanes et les heptachlorodibenzo-*p*-dioxines et furanes. Des hypothèses sur la provenance de ces contaminants seront proposées. Les concentrations maximales furent détectées à des profondeurs variant entre 5 et 8 cm. Contrairement au Saguenay, les résultats de la Baie des Anglais démontrent que les congénères prédominants sont les tétrachlorodibenzo-furanes suivis des pentachlorodibenzo-furanes.

A ces mêmes stations, des crabes, des crevettes, des buccins, des plies, des turbots et des morues furent analysés. Bien que les concentrations soient relativement faibles, le crabe est l'espèce la plus contaminée suivi du buccin, des crevettes et des poissons. Contrairement aux sédiments, les congénères dominants dans les tissus biologiques sont les tétrachloro-furanes. De plus, il existe une excellente corrélation entre le profil de tétrachloro-furanes dans les sédiments et ce même profil pour les crustacés prélevés à la même station.

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BROWN[1] D.J., KENT[2] R.A., CAUX[2] P.Y., WALKER[2] S.

RESEARCH REQUIREMENTS FOR THE DEVELOPMENT OF CANADIAN WATER QUALITY GUIDELINES

The Canadian Council of Ministers of Environment (CCME), Task Group on Water Quality Guidelines, has been developing national water quality guidelines on priority pesticides and industrial chemicals since 1987. These guidelines are based on data and information extracted from a critical view of the existing scientific literature. The guidelines are developed according to strict protocols for minimum data requirements to ensure consistency. Interim guidelines are developed when insufficient information exists to make an informed decision on the development of a specific guideline. At times, no guidelines for priority substances are recommended due to the sparse scientific information available.

A list of priority substances is presented, along with the scientific data required to complete the development of interim water quality guidelines to full guideline status. It is hoped that this list will stimulate interest in the scientific community to provide the research to complete the development.

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BROWNLEE[1] B.G., MacINNIS[1] G.A., DUTKA[1] B.J., XU[2] W., LOCKHART[3] W.L., METNER[3] D.A.

POLYCYCLIC AROMATIC HYDROCARBON ANALYSIS AND ECOTOXICOLOGICAL TESTING OF ATHABASCA RIVER WATER AND SEDIMENT

Water (10 sites) and suspended sediment (8 sites) were collected from the Athabasca River in August, 1990 from Hinton to 200 km downstream from Fort McMurray, a distance of about 1150 km. Organic solvent extracts were analyzed for polycyclic aromatic hydrocarbons (PAH) and subjected to a battery of ecotoxicological tests (Microtox®, nematode and Daphnia). Individual PAH levels in waters were from below detection to 1 ng/L, and in suspended sediment from below detection to 10 ng/g. Waters extracts gave no significant response in any of the ecotoxicological tests. Suspended sediment extracts showed a generally increasing toxic response with distance downstream, the highest being in the area of the oil sands deposits on the lower Athabasca. This was most pronounced in the Microtox® test. PAHs do not account for this response; for example, phenanthrene contributed about 0.1 % of the toxicity of suspended sediment extracts in the Microtox® test. Hepatic mixed function oxidase (MFO) levels in goldeye collected from the lower Athabasca in 1991 showed a similar trend; MFO induction was associated with the oil sands deposits. These results point to (as yet unidentified) substances of natural origin as the source(s) of a significant portion of these ecotoxicological responses.

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BUREAU[1] J., PRUD'HOMME[2] A.M.,
SLOTERDIJK[1] H.H.

RELATIVE MFO ACTIVITIES IN CAGED WHITE SUCKERS EXPOSED TO DIFFUSE CONTAMINATION IN THE ST.LAWRENCE RIVER

Hepatic Mixed Function Oxygenases (MFO's) have been widely used during the past ten years for measuring the biochemical impairment of aquatic organisms exposed to organic contamination. This study evaluated the effects of diffuse contamination on feral fish populations, and focussed mainly on fish hepatic enzymatic activities: ethoxyresorufin -O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH). The purpose of the present project was to give an insight into the different biological factors which could influence the physiological and biochemical responses.

A randomized, replicated factorial experiment was carried out *in situ* in the industrial area of Beauharnois, Québec, contaminated with PCB's, PAH's and heavy metals. In all, 96 White suckers (*Catostomus commersoni*) were captured upstream and downstream the industrial area. The 48 fish from upstream were distributed in six cages (8 fish per cage : 8 m^3 volume and 25 mm mesh-size), three cages being set-up upstream, and the three others being transferred downstream. The 48 fish from downstream received the same treatment. Following an exposure period of 6 to 10 days, hepatic MFO's activities were assayed. This experimental design was carried out three times between June and October 1991.

The kinetics of induction and recovery in a feral fish population exposed to diffuse contamination will be discussed as well as the usefulness of the cage methodology.

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[2] *Analex Inc., Laval (Québec)*

BUCHMAN[1] M.F.

REVIEW AND SUMMARY ON JOINT ACTION TOXICITY TO AQUATIC ORGANISMS

Management of hazardous wastes is increasingly emphasizing the reduction of risk as the measure of progress. For protection of natural resources, the primary vehicule for the assessment of risk, and the basis for risk management decisions, is the Ecological Risk Assessment. The most common approach to Ecological Risk Assessments currently being employed is an adaptation of the indirect risk assessment method commonly used for human health assessments, the Hazard Quotient ratio. This approach assesses risk by comparing exposure levels to some toxicological benchmark value. However, it evaluates risk on an individual chemical basis in chemical-specific ratios. The cumulative, joint action from all of the compounds an organism may be concurrently exposed to is often not evaluated. On occasion, it is assumed that toxicity is additive, and Hazard Quotients are summed to a Hazard index.

This paper will present a review and summary of current literature on joint action, or toxicity of chemical mixtures, with an emphasis on aquatic resources. Results from toxicity tests of binary or complex mixtures are synthesized to provide overall guidelines on chemical interactions. The intent of this review is to provide for risk management decisions based on sound scientific inferences regarding the cumulative risk of joint action, rather than on mere assumptions. A general framework for the interpretation of chemical interaction will also be presented.

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CONSTABLE[1] M.

CHALLENGES IN THE ASSESSMENT OF COMPLEX MIXTURES IN THE CANADIAN ENVIRONMENT

Determining the environmental toxicity of a complex mixture is highly challenging even with the plethora of highly technical instruments and analytical techniques available to environmental toxicologists. The complex mixtures assessed under the Priority Substances List were from many different sources; some are effluents (chlorinated wastewater effluents, pulp and paper mill effluents), others are industrial wastes (creosote impregnated waste materials) and others are commonly produced wastes (waste crankcase oils). Some of the problems posed by complex mixtures include: highly variable composition over time in individual sources and from source to source; difficulty in analyzing the mixture; estimating which components to focus the toxicity assessment on; finding toxicity information specific to the mixture; determining effects of a complex mixture on variable environments; extrapolating effects from other areas and from lab work to the exposed environments; difficulties related to toxicity interactions between individual components of the mixture; and determining what constitutes "exposure" to the mixture in the environment. The approach frequently used is a toxicity assessment on a suite of components typically found in the mixture that were originally suspected as being toxic. This approach has advantages and disadvantages that will be highlighted in this presentation.

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CRETNEY[1] W.J., GOYETTE[2] D.

POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL SAMPLES FROM KITIMAT ARM, B.C.

A survey of PAHs in Kitimat Arm and its marine approaches was first conducted in 1978/79. Over the last few years some additional studies have been done and more are planned for the future. In the case of surface sediments, it has been possible to make tentative comparisons of the concentrations of some PAHs between the earlier and more recent work, although sampling locations and analytical methods differed. Crab samples were collected at five sites in the fall of 1992. Concentrations of individual PAH congeners generally ranged from the detection limit to a few ng/g in muscle tissue. Individual PAH concentrations in crab hepatopancreas were about ten-fold greater, as expected. Both the sediments and crab tissues contained, however, higher than expected and variable concentrations of alkylated aromatic hydrocarbons. This finding may reflect multiple inputs of PAHs in the area.

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DAIGNEAULT[1] M.

MODELE STRATIFIÉ ET ÉCOULEMENT DIFFÉRENTIEL DES DIFFÉRENTS NIVEAUX DE pH DANS LES DÉCHARGES A NEIGES USÉES

Au Canada, c'est au sud du Québec que l'on observe les pluies les plus acides avec un pH moyen de 4.4. Ces pluies acides résultant de l'augmentation des émissions d'oxydes de soufre (SO_2) et d'oxydes d'azote (NOx) par les nombreuses industries américaines sont transportées via les vents dominants jusqu'au Québec. L'accumulation des neiges usées, sur des terrains à proximité de boisé, par les villes de St-Hubert et de Longueuil, a provoqué une augmentation de l'acidité des eaux de surface pendant une courte période de temps lors de la fonte de l'amoncellement de neiges usées. Cette augmentation d'acidité fut néfaste pour la faune herpétologique et aviaire des étangs et ruisseaux des boisés respectifs. Pendant le mois de mai 1992, aucune grenouille, aucun crapaud et aucune espèce de poissons n'a été observé dans les étangs et ruisseaux du boisé de St-Hubert alors qu'au printemps précédent ces espèces étaient présentes en grand nombre avant que le site ne soit touché. La même situation létale a été observée à la limite de Longueuil près de l'autoroute 116. Afin d'expliquer cette situation, des analyses de pH ont été réalisées à des endroits stratégiques; par exemple, dans les cours d'eau et les étangs alimentés par les eaux de fonte provenant des décharges en prenant comme postulat que celles-ci augmentaient le taux d'acidité des eaux de surface.

Au mois de juin 1993, les résultats de trois années de recherche ont été présentés au congrès de l'Ordre des chimistes du Québec et une solution afin de minimiser les impacts sur la faune aquatique a été proposée. Depuis nous avons développé un modèle intégrant les notions de stratification et d'écoulement différentiel des différents niveaux de pH afin d'expliquer ce processus complexe, puisque alétoire, d'acidification des eaux de surface. Ainsi la notion de stratification implique que l'amas de neiges usées n'est pas homogène et qu'on mesure localement différentes valeurs de pH. L'écoulement différentiel ou la fonte des neiges usées est essentiellement d'ordre climatique et est lié à la température. Ainsi, la somme des trois paramètres du modèle, 1) le choc toxique printanier, 2) les pluies printanières acides et 3) l'apport en eaux acides des amas de neiges usées contrôle l'acidité des cours d'eau et est néfaste au développement des oeufs d'amphibiens et de poissons. La limite viable est de 5.0. Sur les deux sites étudiés, pendant une période de trois ans, toute la population d'amphibiens a disparu.

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DESCHAUX[1] P.

LE SYSTEME IMMUNITAIRE DU POISSON PEUT-IL ETRE UN BIOINDICATEUR DE POLLUTION?

L'utilisation du système immunitaire (SI) du poisson comme bioindicateur de pollutions aquatiques est conditionnée par différents facteurs physiologiques et physiques. La connaissance du fonctionnement du SI de poisson doit être approfondie; on n'apprécie qu'une réponse globale de ce système (maladies, production d'anticorps, toxicité cellulaire). Ces paramètres sont variables chez les poissons élevés même dans des conditions identiques; l'évaluation d'une immunotoxicité reste très douteuse à cause de résultats très dispersés.

A cet handicap d'ordre physiologique viennent s'ajouter des problèmes de dépendances thermiques et saisonnières de la réponse immunitaire.

Des travaux de biologie cellulaire sont nécessaires pour préciser l'impact d'un polluant ou d'un stress au niveau de la transduction membranaire lymphocytaire (composition lipidique, canaux ioniques, messagers secondaires).

Le SI du poisson doit être un excellent marqueur de pollution à condition de confronter trois types de résultats :

- Expression de maladies au niveau de l'organisme.
- Expression cellulaire (dosage d'anticorps, étude de cellules toxiques).
- Expression d'un dysfonctionnement intralymphocytaire.

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EXPOSITION AU MERCURE DE BALBUZARDS ÉLEVÉS SUR LES TERRITOIRES DE LA BAIE-JAMES ET DE LA BAIE-D'HUDSON

Cette étude a confirmé, pour la première fois à la Baie-James, l'existence d'un transfert important du mercure depuis le biote aquatique des réservoirs vers les espèces semi-aquatiques qui se nourrissent de poissons. La concentration de mercure dans le sang des jeunes Balbuzards fournit à cet égard une excellente mesure de l'intensité de son transfert vers le chaînon trophique supérieur.

La quantité «totale» de mercure que les aiglons accumulent au cours de leur croissance au nid peut être estimée de façon relativement précise à partir de l'analyse des plumes. Nous avons calculé qu'environ 85% de la charge corporelle en mercure se retrouvait éventuellement dans le plumage des jeunes. Cette accumulation préférentielle du mercure dans les plumes, plutôt que dans les organes internes, dure tout le temps de la croissance du plumage, soit de l'âge de 14 jours à 45 jours environ.

La quantité moyenne de mercure accumulée par les aiglons des réservoirs (à l'ouest de LG4) est en moyenne 6,5 fois supérieure à celle des jeunes élevés en milieu naturel (10,5 mg vs 1,7 mg). Cependant, et bien que la mortalité soit deux fois plus élevée à l'intérieur des nids établis dans les secteurs le plus contaminés, nous constatons somme toute assez peu de conséquence néfaste de cette exposition au mercure. En effet, 69 % des nids actifs de ces secteurs ont connu du succès et ces nichées produisent un nombre de jeunes à l'envol ($\bar{x}$ = 1,6 ± 0,7; n = 20) qui est à peine plus bas que celui enregistré aux autres stations situées sur les réservoirs ($\bar{x}$ = 1,9 ± 0,9; n = 35) ou en milieu naturel ($\bar{x}$ = 2,0 ± 0,8; n = 98).

La croissance du plumage fournit un excellent exutoire qui protège efficacement les aiglons des effets nocifs du méthylmercure. Les problèmes toxicologiques pourraient cependant être beaucoup plus sérieux après l'envol, une fois la croissance des plumes terminée. Selon la durée du séjour pré-migratoire des jeunes à proximité des sites de nidification, il pourrait y avoir une accumulation importante de mercure dans les organes vitaux.

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DILLON[1] T.M.

A FRAMEWORK FOR THE ENVIRONMENTAL RISK ASSESSMENT OF CONTAMINATED SEDIMENTS

Sediments generally act as sinks for chemicals released into the aquatic environment. Consequently, many contaminated sites contain sediments with substantial levels of chemicals. A framework for evaluating the environmental risks associated with these contaminated sediments is presented. The framework is consistent with the National Academy of Sciences (NAS) 1983 human health risk assessment paradigm as well as recent proposals by USEPA and NAS for evaluating ecological risks. The framework consists of four elements; Initial Evaluation, Exposure Assessment, Effects Assessment and Risk Characterization. Initial Evaluation is a scoping process including assessment and measurement endpoints, receptors of concern, QA/QC, appropriate reference sediment, etc. Exposure Assessment is a 2-tiered process for determining the fate and spatial/temporal distributions of sediment-associated contaminants relative to the receptor of concern. Effects Assessment is the process of establishing the strength or magnitude of the chemical hazard in sediments by evaluating exposure-response or dose-response relationships. This framework considers effects on both human and non-human receptors of concern. Sediment bioassays, recommended for most non-human receptors, can address the Byzantine mixtures of conventional, exotic or unknown chemicals embedded in a geologically complex matrix. Qualitative and quantitative uncertainty analyses are conducted during the last stage of the framework; Risk Characterization. Here, the ecological importance of projected risks to non-human target species is discussed vis-à-vis the initial assessment endpoints.

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THE PARTITIONING OF MERCURY IN THE SOLID COMPONENTS OF A FOREST SOIL AND SEDIMENTS FROM A HYDROELECTRIC RESERVOIR

Soil and sediment samples collected along a transect at the LG-2 reservoir and watershed were subjected to a sequential extraction procedure to determine the distribution of mercury in three operationally-defined solid compartments: mercury associated with organic carbon (1 N NaOH-extractable), mercury associated with reactive Fe/Mn minerals (1 N HCl-extractable), and the mercury remaining in the solid (clays and sulphides) residue. Laboratory results indicate that up to 80 % of the mercury in the O-horizon of forest soils and flooded forest soils and up to 85 % of the mercury in lake bottom sediments is bound to the NaOH-extractable organic carbon fraction. It was also noted that mercury is preferentially associated with partially degraded organic matter located at the bottom of the O-horizon.

In the mineral accumulation zone where the organic content is low, 50-70 % of the total mercury was found associated with reactive Fe/Mn minerals. In contrast, the flooded soil contains almost no reactive Fe and mercury concentrations are low (<10 ppb). We propose that upon inundation, mercury is liberated from this compartment and is either dispersed into the pore waters or readsorbed by another substrate. Analyses of the final residues suggest that there is an enrichment of mercury in the clay fraction above the B-horizon in a flooded soil, while sulphide mineralization may play a role in sequestering mercury in lake sediment.

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LIVING LUMINESCENT BIOSENSORS TO MONITOR AQUATIC POLLUTANTS AND DETERMINE THEIR UNDERLYING MECHANISMS OF TOXICITY

The rapid and inexpensive determination of specific contaminants remains a constant problem in aquatic ecosystems. We have been using luciferase (lux) gene fusions to create living biosensors that can identify the bioavailable presence of common pollutants in aquatic ecosystems. Moreover, due the gene tagging involved in their construction, we are able to identify chromosomal genes whose expression is induced by these compounds, and thus explore the genetic basis on sensitivity (or resistance) displayed by individual organisms or species.

Our biosensors consist of clones of the genetically well-characterized bacterium E. coli which contain the luciferase-encoding luxA,B genes (minus their promoter) fused to different chromosomal genes of the bacterium, such that luciferase expression is controlled by the bacterial gene into which the luxA,B genes have been inserted. These clones will glow (luminesce) due to the fact that the bacterial gene's expression is induced when the bacterium senses stress from the presence of a particular toxic agent. In addition, the use of a portable luminometer allows our clones to be used in the field. We currently have clones that can detect aluminum, arsenic/antimony, nickel, selenium and tributyltin, and are isolating new clones for other common and troublesome toxic compounds. The genes (and their protein products) which are regulated by exposure to these compounds are being molecularly cloned, and their DNA sequenced, in order to determine the cellular response to environmental pollutants, and conservation of these genes in other organisms.

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INDICATEUR CHIMIOTOX POUR LES EFFLUENTS INDUSTRIELS

Le Chimiotox est un modèle permettant l'évaluation chimio-toxique des effluents industriels. L'Équipe d'Intervention Saint-Laurent l'a développé pour mesurer et rendre accessible au grand public les progrès accomplis au niveau de la réduction des toxiques des cinquante industries prioritaires du Plan d'action Saint-Laurent. Il est basé sur la caractérisation physico-chimique des contaminants. Ensuite, une pondération toxique est appliquée aux différents contaminants de façon à les ramener sur un dénominateur commun. Le produit de la charge polluante par le facteur de pondération toxique donne des unités Chimiotox. Les unités Chimiotox individuelles de chaque contaminant sont additionnées pour définir l'indice Chimiotox. L'intégration des résultats peut se faire par industrie, par groupe de contaminants, par secteur industriel ou par secteur géographique. Le modèle Chimiotox se révèle un outil polyvalent qui permet d'identifier les substances toxiques prédominantes, d'unir les résultats en une seule base de données et d'obtenir une image d'ensemble des rejets toxiques.

L'indice Chimiotox ne tient compte que des substances toxiques prioritaires que l'on retrouve dans les effluents industriels. Il ne tient compte, ni de la capacité du milieu récepteur, ni de l'acidité, ni de l'effet des substances les unes sur les autres, ni des paramètres conventionnels comme les MES, DBO, DCO, etc. Le modèle Chimiotox a été expérimenté avec les données du Plan d'action Saint-Laurent. Grâce à ce modèle, nous avons pu démontrer que les cinquante industries du PASL avaient réduit leurs rejets toxiques de plus de 75 % de 1988 à 1993.

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ECOLOGICAL RISK ASSESSMENT FOR THE TERRESTRIAL ENVIRONMENT

This paper outlines the basic concepts and guiding principles of terrestrial ecological risk assessment (ERA) and provides examples of how these concepts and principles can be applied in predictive or retrospective evaluation of environmental stressors. Data requirements for ERA exposure and toxicological analyses are identified and basic methods for exposure and toxic characterization are discussed. The use of hazard quotients, Monte Carlo simulations, and terrestrial population models in risk analyses also is discussed. Current limitations of the state-of the practice and future research needs are identified.

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PREDICTING EFFECTS OF CHLOROBENZENES ON BENTHIC BIOTA : CONSTRAINTS AND APPROACHES

Ecological assessments under the Canadian Environmental Protection Act (CEPA) Priority Substances Assessment Program require a multimedia approach to determine whether substances may cause harmful effects to the Canadian environment. Estimating effects to benthic biota from exposure to priority substances in sediments poses a distinct challenge because of a lack of data on the bioavailability of substances in sediments, and a lack of bioassay data. Several modelling approaches were used to predict the effects of chlorobenzenes in sediment on benthic biota for freshwater and marine environments : 1) equilibrium partitioning (EP) approach, 2) apparent effects threshold (AET) approach, and 3) critical body ratio (CBR). The results of these modelling approaches and their implications for the ecological assessments for chlorobenzenes ranging from mono- to hexachlorobenzene will be discussed.

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ENGEL[1] D.W.

METALLOTHIONEINS AND TRACE METAL PARTITIONING IN THE ATLANTIC OYSTER, Crassostrea virginica

Oysters' ability to accumulate trace metal, particularly the transition metals (Cd, Cu, Zn, and Hg), from food and waters has been well documented. There is little information, however, on how these metal interact to affect whole animal retention, partitioning, and binding to metallothionein (MT). In our laboratory oysters have been exposed to Cd and Cu both alone and in combination, and significant effects have been demonstrated on subcellular partitioning. These studies showed that Cu can displace Cd from MT, but the Cd is not lost from the animal. Two metal-binding protein peaks have been separated by gel chromatography (< 12K and 24K daltons). The lower molecular weight protein peak has been characterized as MT, consisting of two isoproteins, but the higher molecular weight protein peak appears to be an aggregation of metal-binding proteins, some of which may be MT or an MT dimer. In environments where Cu or Cd contamination is present, the MT binds primarily these metals and almost no Zn. In noncontaminated systems MT binds primarily Zn and only one of the two isoMTs appears to be involved. In the presence of either Cu or Cd, both isoproteins bind Cd and/or Cu. Differences have been noted between northern and southern populations of oysters with regard to cytosolic metal partitioning. The importance of these observations to the use of the oyster as a sentinel organism will be discussed.

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ENGEL[1] D.W., EVANS[1] D.W.

MERCURY BIOACCUMULATION BY FISH AND CRABS IN A TEXAS BAY : A MODELING APPROACH

Lavaca Bay, Texas was contaminated with mercury for over ten years by the releases from a chlor-alkali plant located on the Bay. A portion of the Bay has been closed to commercial and recreational fishing, because some of the fish and crabs exceed the FDA action limit for methylmercury (1 PPM). Models were developed describing the food web pathways involved in the accumulation of methylmercury by fish, black drum, *Pogonias cromis*, red drum, *Sciaenops ocellatus*, and blue crab, *Callinectes sapidus*. The models predict the accumulation of mercury from sediments through intervening prey organisms. Much of the data used to quantify the model were taken from the literature, since only a few direct measurements of mercury and methylmercury concentrations in fish and crabs were available (Texas Department of Health data). The model predictions of mercury accumulation by fish and crabs from their prey are dependent upon a simplified bioenergetic equation, incorporating estimates of weight specific feeding rates, growth, and methylmercury excretion. These predictions are coupled with species specific patterns of feeding on prey of different methylmercury concentrations. The predictions that are generated by these models fall within the range of methylmercury concentrations actually observed in the target fish and crab species.

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ENGLAND[1] S.

APPLICATIONS OF HUMAN GENETICS TO AQUATIC TOXICOLOGY

Biomarkers have been successfully applied in the medical field for the identification of individuals at risk of disease or in the pre-symptomatic stages of disease. Now, biomarkers are being applied in the evaluation of human health damage due to exposure to environmental or occupational contaminants and to develop guidelines for acceptable risk in toxic chemical emissions or in the remediation of contaminated sites. This application depends on the use of biomarker index values derived from laboratory animal studies or from epidemiological studies of accidentally exposed human populations to understand the relationship between an observed biomarker value and the level of health damage. Problems with using biomarkers in ecosystem monitoring include : uncertainties in extrapolating lab animal or human study-derived indices to values found in species which may be impossible to study systematically due to situational constraints; impracticalities in applying sample collection or sample storage methodologies in the field; and most importantly, problems in evaluating which biomarker and indicator species to evaluate in order to best estimate the health of the ecosystem. In this paper, the application of biomarkers to ecosystem health evaluation is discussed with particular reference to measurements of genetic damage resulting from environmental contamination by toxic chemicals. The relevance of humans as an indicator species is also examined.

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LE TEST JAYLET (OU TEST MICRONOYAU TRITON) : UN NOUVEL OUTIL POUR LA DÉTECTION IN VIVO DE LA GÉNOTOXICITÉ DES EAUX DOUCES

Un test micronoyau révélant à l'échelle cellulaire certaines aberrations chromosomiques a été mis au point au Centre de Biologie du Développement de l'Université Paul Sabatier de Toulouse. Ce test, applicable en routine, permet de déceler les effets clastogènes et/ou aneugènes des substances mutagènes présentes dans l'eau. Il a d'abord été réalisé sur les érythrocytes du sang circulant des larves aquatiques du triton *Pleurodeles waltl*, puis sur ceux des larves de l'Axolotl (*Ambystoma mexicanum*) et du crapaud xénope (*Xenopus laevis*). Ce test permet de détecter la génotoxicité d'une substance chimique seule ou en association et celle d'échantillons d'eau potable ou en cours de potabilisation. Le test Jaylet (du nom de son inventeur*) a fait l'objet d'une norme AFNOR (T90-325, 1992).

Un centre d'intérêt majeur de ce test réside dans le fait que sa sensibilité permet son utilisation en écotoxicologie. Il permet d'apprécier le pouvoir génotoxique global d'une eau polluée brute, où interviennent les effets de synergie ou d'antagonisme éventuels entre les différents micropolluants d'un mélange complexe; il prend en compte les phénomènes de bioaccumulation, d'excrétion et de métabolisation spécifiques des organismes supérieurs. Il a été appliqué à plusieurs types de rejets industriels (chimiques, pétrochimiques, pétroliers, mégissiers, papetiers, métallurgiques, effluents autoroutiers, etc...) et a révélé la génotoxicité de certains d'entre eux. Le test Jaylet a désormais toute sa place dans les batteries d'approche de la génotoxicité des milieux complexes.

* Décédé en 1989

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ANALYSE DES FACTEURS BIOTIQUES ET ABIOTIQUES INFLUENÇANT LA BIOACCUMULATION DES MÉTAUX LOURDS CHEZ BITHYNIA TENTACULATA (MOLLUSCA : GASTROPODA) DU LAC SAINT-LOUIS

Plusieurs facteurs influencent l'incorporation et la bioaccumulation des métaux par les organismes aquatiques, comme la répartition des métaux dans les différentes phases géochimiques des sédiments, la biodisponibilité des métaux ainsi qu'une quantité de facteurs environnementaux (matière organique, acidité, dureté, ...). Dans le but d'évaluer le potentiel des gastéropodes pour le développement d'indicateurs de la qualité du milieu, nous avons mis en relation les concentrations de métaux dans les gastéropodes, avec celles retrouvées dans les divers compartiments biologiques (seston, périphyton, macrophytes) ainsi que plusieurs facteurs physico-chimiques de l'eau et des sédiments. Nous sommes arrivés à élaborer des modèles de prédiction des concentrations de Cd, Cr, Fe, Mn et Zn dans les gastéropodes, à l'aide de plusieurs variables (modèles de régressions multiples), qui réussissent à expliquer de 55 % à 94 % de la variance. Dans certains cas, la taille des organismes (juvéniles ou adultes) ainsi que la période d'échantillonnage (juillet ou août) ont apporté plus d'explication à nos modèles. Aucune relation n'a pu être établie dans le cas du Cu, Ni et Pb.

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METHYL-MERCURY IN POREWATERS OF AN ANOXIC MARINE SEDIMENT : SAGUENAY FJORD

Methyl-mercury, $CH_3Hg(II)^+$, the main form of mercury in fish, is more toxic than inorganic mercury. Until now, it was assumed that methyl-mercury production resulted from strong bacterial activity in contaminated and organic-rich surface sediments. The distribution of $CH_3Hg(II)^+$ in marine sediments and its flux at the sediment-water interface remain unknown.

Dissolved $CH_3Hg(II)^+$ was analyzed in porewaters extracted from Saguenay Fjord sediments sampled at three different stations in the landward basin. These sediments are characterized by high mercury concentrations due to industrial loading from a chlor-alkali plant and a very thin oxidized layer (<1 cm) below which strongly reductive conditions were detected. $CH_3Hg(II)^+$ concentrations in porewaters were relatively high below the redox interface and reached up to 10 ng Hg L^{-1} (50 pM). Profiles also indicated that, despite a high concentration gradient, little $CH_3Hg(II)^+$ escapes to the overlying water column because the oxidized sediments at the interface seem to act as an efficient boundary. In the oxic zone, $CH_3Hg(II)^+$ may be sorbed by fresh organic matter and iron oxyhydroxides or subjected to catalyzed demethylation by Fe-Mn oxides.

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ÉTUDE DE LA CINETIQUE D'ACCUMULATION DU CADMIUM PAR UNE MOUSSE AQUATIQUE, FONTINALIS DALECARLICA SCHIMP. EX. B.S.G., EN LABORATOIRE

Nous présentons les résultats d'études en laboratoire sur la cinétique d'accumulation du cadmium par une mousse aquatique, *Fontinalis dalecarlica Schimp.* ex. B.S.G. Les concentrations nominales reconstituées *in vitro* sont de 0 ppb (témoin), 10 ppb et 50 ppb. Les concentrations réelles au début des expériences étaient sous le seuil de détection pour le témoin ($<0{,}6$ ng l^{-1}), de 12,0 $\mu g.\ l^{-1}$ et 45,4 $\mu g.\ l^{-1}$ de Cd. Les expériences durent 48 heures, les études sont effectuées en système statique. A cause du grand pouvoir accumulateur des mousses, les concentrations de Cd dans l'eau ont chuté très rapidement, et se sont épuisées (sous le seul détectable). Malgré tout, les mousses aquatiques ont accumulé le Cd, les résultats sont satisfaisants étant donné les conditions des expériences. Pour une contamination de départ de 12,0 $\mu g.\ l^{-1}$, le maximum de Cd bioaccumulé par les mousses est de 46,53 $\mu g.\ l^{-1}$, ce phénomène est observé après 8 heures d'intoxication. La bioconcentration de cette bryophyte, à une contamination de départ de 45,4 $\mu g.\ l^{-1}$, atteint un maximum de 141,12 $\mu g.\ l^{-1}$ de Cd après 48 heures d'exposition.

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BIPHÉNYLS POLYCHLORÉS DANS LE PANICULE ADIPEUX DES BALAENOPTÉRIDES DU GOLFE DU SAINT-LAURENT, QUÉBEC: ÉCHANTILLONNAGE PAR BIOPSIES ET SUR CARCASSES ÉCHOUÉES

Peu d'information est disponible sur la contamination des rorquals du Saint-Laurent. L'analyse des BPCs dans le panicule adipeux de quatre espèces de rorquals visitant cette région ont été analysés. L'échantillonnage du panicule adipeux des rorquals a été effectué par biopsies et sur des carcasses échouées sur les rives du golfe. Les BPCs ont été déterminés par chromatographie capillaire en phase gazeuse munie d'une colonne de silica fusionnée. 18 congénères de BPC de concentration totale variant entre 1 et 8ppm (masse lipidique) ont été décelés chez les rorquals. Les différences possibles entre les espèces et l'efficacité de la méthode biopsidique seront discutés.

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GEARING[1] J.N., GEARING[1] P.J., NOEL[1] M., SMITH[2] J.N.

POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS OF THE ST.LAWRENCE ESTUARY

Elevated concentrations of polycyclic aromatic hydrocarbons (PAH) characteristic of combustion processes have been previously reported in the Saguenay Fjord and Baie des Anglais, and associated with the historical record of discharges by aluminum refineries. We analyzed PAH in sediments from the Saguenay Fjord (four cores) and the Lower St.Lawrence Estuary (15 locations). A dated Saguenay core contained total combustion PAH at concentrations of 1.9 to 160 μg/g which varied with time and sediment displacements. In the Lower Estuary, transects showed elevated levels of PAHs (up to 8.9 μg/g) near the north shore where a refinery is located. In the deep Laurentian Trough, however, PAH concentrations averaged around 1 μg/g (range 0.69 to 1.6 μg/g) and showed no apparent geographic or temporal trends, indicating that the refineries have little influenced these sediments. The ratio of benzo(a)pyrene (a combustion PAH) to perylene (a PAH of similar physio-chemical properties with natural sources) was used to trace PAH from refineries by minimizing variability due to sedimentary grain size and organic carbon content. A series of PAH compounds derived from resin acids of terrestrial plants (norabieta-tetraene, norabieta-triene, tetrahydroretene and retene) was also prominent in the upper Saguenay core and may reflect the pulp and paper industry.

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GÉLINAS[1] S., TESSIER[1] L., PARIS[1] B., PAZDERNIK[1] L.

ÉTUDE DE L'ACCUMULATION DE MÉTAUX ESSENTIELS (Ca, Cu, Fe, Mg, Mn, Zn) CHEZ LE GASTÉROPODE VIVIPARUS GEORGIANUS (LEA)

Nous avons étudié les processus d'accumulation de métaux essentiels chez le gastéropode de faible longévité *Viviparus georgianus* (Lea) en fonction de la hauteur des individus, laquelle reflète assez fidèlement l'âge du gastéropode. Les concentrations des six métaux ont été mesurées par spectrophotométrie d'absorption et d'émission atomique. Les résultats démontrent que les concentrations en Mg, Mn et Zn diminuent en fonction de la hauteur des individus ($R^2>0,60$). Cette relation est moins évidente pour les concentrations en Ca et en Cu ($R^2<0,40$). Par contre, les quantités de Ca et Cu par individus augmentent en fonction de la hauteur des spécimens ($R^2>0,70$), mais cette relation est moins significative pour les quantités de Mg, Mn et Zn par individus ($R^2<0,60$). L'accumulation du Fe ne semble pas influencée par la hauteur des gastéropodes, d'autres facteurs pourraient être impliqués. Les patrons d'accumulation des métaux étudiés nous permettent d'établir deux groupes de métaux (Ca, Cu, Fe et Mg, Mn, Zn), lesquels semblent être reliés à leur activité métabolique au sein des gastéropodes.

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CILIATED PROTOZOA AS TEST ORGANISMS FOR TOXICITY BIOASSAYS

Ciliated protozoa are important as food for higher trophic levels and as recyclers and remineralizers of organic material in the benthic and pelagic realms of aquatic ecosystems. The characteristics of these easily cultured and rapidly growing protozoa are well known. Given the proliferation of new microbial bioassay technologies, ciliates have become useful subjects in toxicological research, and more recently, as test organisms for bioassays. Ciliate bioassays have been developed by a number of researchers worldwide, over the past 20 years. These bioassays have utilized a variety of test organisms, and, as is the case with other toxicological tests, a variety of lethal and sublethal indicator parameters. Among the indicator parameters are : morphology, mortality, growth, motility, respiration, genotoxicity, and chemotaxis. This paper summarizes and reviews the recent literature on bioassays using ciliates, and discusses them in terms of their practicability and feasibility for routine monitoring of environmental toxicants.

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GILRON[1] G.L., LYNN[2] D.H., SCROGGINS[3] R.P.

DEVELOPMENT AND VALIDATION OF A NEW, RAPID AND ECONOMICAL PROTOZOAN BIOASSAY FOR PULP AND PAPER EFFLUENTS

This study is part of an ongoing research program aimed at developing a standard protozoan bioassay to complement Environment Canada's Biological Test Method Development program. The study has focused on the development of a sublethal bioassay using ciliated protozoans as test organisms. Based on earlier work by Roberts and Berk (1992), the bioassay utilizes the chemotactic response exhibited by ciliates when exposed to reference toxicants and whole effluents. During the previous phase, a working protocol was developed and tested with several reference toxicants, and with various industrial effluents (Gilron et al., 1991). Comparisons with standard fish and crustacean tests indicate that the bioassay has promise for monitoring pulp and paper effluents. The current phase of the study has mainly involved the technical refinement of the protocol, including enhancements to cell enumeration methods, pretreatment of cell cultures to sensitize the organisms to toxicants, and varying the exposure time of the test. Future work will involve an interlaboratory comparison («round-robin» tests) of the bioassay, and an intralaboratory comparison of the ciliate bioassay with other standard sublethal bioassays.

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SEDIMENTARY RECORD ISOTOPIC COMPOSITION AND BURDEN OF INDUSTRIAL LEAD IN THE LAURENTIAN TROUGH SEDIMENTS

The depth distribution of the concentration of lead has been determined in sediments from seven box cores collected along the longitudinal axis of the Laurentian Trough, Lower St.Lawrence Estuary. In one of these cores and in deep sediment samples collected with gravity and piston corers, we measured the concentration of lead and its stable isotope composition in three fractions obtained by sequential extractions. The results reveal three isotopically-distinct types of lead for this environment : two from natural sources and one from recent industrial pollution. The presence of two types of natural lead is attributed either to differential weathering of the magmatic rocks of the Canadian Shield or to the different physiographic regions within the drainage basin of the St.Lawrence River and Estuary. Industrial lead has an isotopic composition ($^{206}Pb/^{207}Pb = 1.151$; $^{206}Pb/ ^{208}Pb = 0.4985$; $^{206}Pb/^{204}Pb = 21.0$) typical of that of the lead found in urban aerosols in Canada. The total burden of this type of lead in sediments deposited below 200 m in the entire Lower St.Lawrence Estuary is of the order of 16 500 x 10^3 kg, on average about 175 x 10^3 kg annually since the beginning of the present century. The sedimentary lead record observed at each station indicates, however, a recent decrease in the rate at which lead has been added to this coastal marine environment. Lead having an isotopic composition typical of American industrial lead found in the atmosphere does not have to be invoked to rationalize our observations on the Laurentian Trough sediments; it does not contribute more than 10 %.

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IDENTIFICATION OF THE TOXIC CONSTITUENTS (TIE) IN A BCTMP MILL EFFLUENT

Slave Lake Pulp Corporation commissioned a 350 ADMT/D bleached chemi-thermo-mechanincal pulp (BCMTP) mill in late 1990. Included in the design was the application of processes which would conserve water (18m^3 of water per ADT of pulp produced) along with an extended aeration activated sludge system to treat process wastewater. The dissolved constituents in the wastewater are concentrated as a result of the mill's water conservation practices. Although the effluent is not acutely toxic, there were some questions about whether or not these practices influence chronic toxicity. A study was conducted to first characterize and then identify any effluent constituents that cause chronic toxicity.

The effluent from the Slave Lake mill is not acutely toxic to trout, *Daphnia*, fathead minnow, and the luminescent bacterium *Photobacterium phosphoreum*. However, the effluent impaired reproduction in *Ceriodaphnia* (IC_{50} = 13 %) and inhibited growth of the green alga *Selenastrum capricornutum* (IC_{50} = 4 %). This pattern of effluent toxicity was established with additional sampling and testing of the effluent over a two month period.

The effluent was then fractionated to identify the toxic constituents. The toxicity identification evaluation or TIE consisted of three phases; characterization, identification, and confirmation. Zinc was identified as the toxic constituent inhibiting alga growth. Copper and to a lesser extent sodium were responsible for the observed effects on reproduction in *Ceriodaphnia*. We will review the fractionation procedures, particularly those procedures recommended in the USEPA TIE manuals that were ineffective in separating the inorganic toxicity.

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ACCUMULATION DE DEUX MICROPOLLUANTS ORGANIQUES PAR L'ALGUE PICOPLANCTONIQUE SYNECHOCOCCUS : EFFETS DE LA MATIERE ORGANIQUE DISSOUTE ET DU pH

Le but de notre étude est de préciser l'importance de la concentration en matière organique dissoute naturelle (MOD), et du pH, sur la bioaccumulation de micropolluants organiques: le pyrène (PYR), hydrocarbure aromatique polycyclique, et le 2-2'5-5'-tétrachlorobiphényle (TCB), biphényle polychloré. L'organisme étudié est l'algue picoplanctonique *Synechococcus leopoliensis*. La MOD utilisée est un acide humique aquatique de référence. Les essais ont été réalisés à pH 7.3 et 4.3. Le principe de l'essai est d'exposer les algues à une concentration dissoute constante (forme libre) de PYR ou de TCB marqués au Carbone-14, tout en augmentant la concentration en acide humique, et donc la concentration en micropolluant associé à la MOD. Des sacs à dialyse (seuil de coupure de 1000 Dalton) sont remplis de solutions d'acide humique (0,2, 5, 20 mg/L) et placés dans un aquarium contenant une solution de micropolluant. Après diffusion du micropolluant dans les sacs et atteinte d'un équilibre des concentrations (concentration libre d'environ 1 μg/L), les algues sont ajoutées à 10^7 cellules/mL. Au bout de 48 heures, la quantité de polluant liée à *Synechococcus leopoliensis* est déterminée par différence avec un contrôle ne contenant pas d'algues, tandis que la quantité liée à la MOD est déterminée par différence avec un contrôle ne contenant pas de MOD. Les résultats montrent que la quantité de MOD n'influence pas de façon significative la bioaccumulation du PYR ou du TCB, à condition que la concentration dissoute de la forme libre soit maintenue constante. Par contre le pH influe sur la quantité de contaminant associée à la MOD qui passe de 30 % à 42 % pour le TCB et de 41 % à 54 % pour le PYR entre pH 7.3 et 4.3 (pour 20 mg/L DOM). Il influe également sur la quantité de polluant liée aux algues qui passe de 36 % à 44 % pour le TCB et de 32 % à 37 % pour le PYR entre pH 7.3 et 4.3.

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GREENE[1] J.C.

SELECTION OF TOXICITY BIOASSAYS BASED UPON ORGANISM SENSITIVITY

Bioassessment of contaminated surface water and ground water will provide a direct response to complex chemical and physical sample dynamics and an estimate of the bioactive components in a waste site sample. The bioassessment approach is more direct, realistic and toxicologically meaningful than chemical measurements and calculations of predicted LC_{50} values based on chemical concentrations.

Many aquatic organisms have been proposed for testing, but few have been used in standardized protocols. The sensitivities of test organisms used in standardized tests, such as, bacteria (*Photobacterium phosphoreum* = Microtox® and *Escherichia coli* = Toxichromotest), macro invertebrates (*Daphnia magna*) and fish (*Pimephales promelas*), were compared, using split samples, in more than 200 filtered site sample solutions. The test solutions were elutriates prepared from soil or sediment, ground water, and surface water.

This presentation will discuss the comparative results from these tests and the importance of using a battery of toxicity test procedures.

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ÉVALUATION DE L'EXPOSITION DES PECHEURS SPORTIFS QUÉBÉCOIS AU MÉTHYLMERCURE : RÉSULTATS PRÉLIMINAIRES D'UNE ÉTUDE-PILOTE

La consommation de poisson et fruits de mer constitue la principale source d'exposition au méthylmercure (MeHg) pour la population générale. Les pêcheurs sportifs sont donc susceptibles d'être particulièrement exposés. Afin d'explorer diverses avenues pouvant permettre d'évaluer cette exposition, une étude-pilote a été réalisée auprès de pêcheurs sportifs ($n=94$) de la région du Lac St-Pierre. L'étude a permis de mettre en relation la consommation saisonnière de différentes espèces de poisson et les concentrations de MeHg dans les cheveux.

Les concentrations de mercure varient de 0,20 à 8,97 µg/g [R_1 moyenne$=0{,}77$ ($IC_{95\%}$ 0,65;0,90), $R_2=1{,}19$($IC_{95\%}$ 0,83; 1,54), $R_3=1{,}52$ ($IC_{95\%}$0,91;2,14)]. La consommation moyenne totale de poisson et de fruits de mer est de 7866 g par année ($IC_{95\%}$ 6467,64; 9264,45), dont 4426 g ($IC_{95\%}$ 3543,18;5309,76) sont constitués de poisson de pêche sportive. Il existe une corrélation significative entre la quantité consommée et le taux de mercure retrouvé dans les cheveux et ce, pour plusieurs espèces. C'est pour la consommation de perchaude ($r=0{,}74, p<0{,}005$), principale espèce consommée dans cette région, que cette évidence est la plus constante.

Cette étude nous a démontré que les pêcheurs sportifs du Lac St-Pierre, consommant leurs prises, ont une consommation de poisson supérieure à celle de la population générale canadienne. Cependant, les niveaux moyens de mercure retrouvés dans les prélèvements de cheveux se situent en deçà des concentrations maximales recommandées par les organismes nationaux et internationaux de santé.

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GÉOCHIMIE DU Hg ET DU Pb DANS DES SOLS FORESTIERS AVANT ET APRES INONDATION

Des sols forestiers (podzols, sols à gley et sols organiques) et leurs équivalents inondés furent échantillonnés dans la région des réservoirs de LG-2 au Nord et de Cabonga au Sud. Les teneurs en carbone, azote, oxydes de fer et de manganèse, mercure et plomb furent analysées selon une maille centimètrique. Dans les podzols, les hautes teneurs en métaux lourds sont strictement confinées à l'horizon humique, sous la couche végétale vivante de surface et nettement séparées des oxydes de fer accumulés dans l'horizon illuvié. Au contraire, dans les sols à gley et dans les sols organiques, le Hg et le Pb sont lessivés tout comme les oxydes de fer jusqu'aux niveaux variables de la nappe phréatique.

Suite à l'inondation des sols, la dégradation bactérienne de la matière organique se manifeste par une rapide baisse du Eh, mais n'occasionne pas une diminution détectable des teneurs en carbone organique particulaire. Dans les podzols et les sols organiques inondés, le Hg et le Pb semblent aussi rester stables, à la fois en concentration et en profondeur, et ce malgré une remobilisation intense des métaux de transition vers la surface. Au contraire, dans le sol à gley inondé, le Hg et le Pb ont subi une remobilisation partielle vers la surface conjointement avec le fer. Dans tous les cas étudiés, la contamination des organismes des réservoirs par le Hg ne correspond pas à une perte significative, après mise en eau, du Hg initialement présent dans les sols.

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GUAY[1] I.

LES CRITERES DE QUALITÉ DE L'EAU AU QUÉBEC : DÉFINITION ET APPLICATION

Le ministère de l'Environnement du Québec (MENVIQ) a récemment rendu public deux documents concernant les critères de qualité de l'eau qu'il entend utiliser pour des fins de suivi de qualité et de protection des usages du milieu récepteur aquatique. Le MENVIQ a ainsi retenu des critères de l'eau pour plus de trois cents contaminants, pour la plupart des substances toxiques, ainsi qu'une méthodologie permettant de calculer un critère pour les substances potentiellement nuisibles qui n'en possèdent pas encore.

Les critères de qualité de l'eau reflètent la meilleure information disponible jusqu'à présent sur les effets délétères d'un contaminant. L'utilisation de ses valeurs ne peut se faire sans l'application constante du «meilleur jugement professionnel». Il est donc nécessaire que leurs utilisateurs connaissent les grandes lignes de leur fondement ainsi que leur cadre d'application.

Dans ce contexte, le présent exposé a pour but de donner les définitions des critères, d'expliquer leur fondement scientifique et surtout d'identifier leurs limites d'application tant techniques qu'administratives. L'emploi qu'en fait actuellement le MENVIQ sera aussi abordé.

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HAGUENOER[1] J.M.

BIOMARQUEURS POUR L'ÉVALUATION DE LA SANTÉ HUMAINE

L'utilisation des biomarqueurs pour évaluer les risques pour la santé est déjà très ancienne en ce qui concerne les hommes exposés par leurs activités professionnelles. Elle a apporté des arguments suffisamment efficaces pour qu'ils fassent l'objet de réglementations dans de très nombreux pays industrialisés. L'hygiène industrielle et la Médecine du Travail ont ainsi permis de grands progrès dans la prévention. Malheureusement, la transposition aux populations exposées par le fait de l'environnement n'a pas été systématique et s'avère même exceptionnelle dans certains pays industrialisés.

Les marqueurs biologiques utilisés dans l'évaluation des risques pour l'homme, peuvent être classés en plusieurs catégories, en fonction de la précocité de leurs modifications :

1- La dose externe, importante à évaluer car elle doit toujours être mise en corrélation avec la dose interne et les effets ou la dose externe.
2- La dose interne qui est le meilleur reflet de l'imprégnation d'un individu et qui intègre toutes les voies d'entrée des toxiques. Il peut s'agire du toxique lui-même soit de l'un de ses métabolites. Le choix du milieu biologique (sang, urine, cheveux, dents, etc...) est fonction des corrélations recherchées avec les effets.
3- Les marqueurs précoces de toxicité, parmi lesquels il faut inclure :
    - les *facteurs de susceptibilité individuelle* (génotypage, maladies, alcoolisme, tabagisme notamment)
    - les *marqueurs biologiques* : leur choix doit se faire en fonction de la précocité par rapport à d'autres modifications, de la réversibilité de l'effet (exemples du plomb, du cadmium, des solvants), et des possibilités techniques et financières pour des dépistages systématiques. Les marqueurs précoces pour les cancers ne sont pas validés actuellement, mais leur intérêt dans des enquêtes épidémiologiques est croissant. Nous donnerons un exemple

d'étude de validation en cours.

- les *tests de mutagénèse* : ils font partie des marqueurs biologiques précoces mais certains sont particulièrement intéressants comme l'analyse des adduits à l'ADN car, avec cette technique, nous disposons d'une double information avec une seule analyse puisqu'elle intègre la dose et l'effet.
- les *marqueurs physiologiques* : ils sont plus ou moins intéressants, car parfois difficiles à mettre en oeuvre dans la pratique. Des techniques comme les vitesses de conduction nerveuse (pour certains solvants, le plomb ou l'oxyde d'éthylène) ou les électrocardiographies pour les cardiotoxiques et surtout les épreuves fonctionnelles respiratoires sont de plus en plus utilisées en milieu professionnel. Il existe néanmoins des limites tant dans leur utilisation que dans l'interprétation de leurs résultats.
- les *tests psychotechniques* qui sont déjà très utilisés pour évaluer les risques précoces des enfants exposés au plomb. Les interférences dans les résultats sont considérables et leur utilisation nécessite beaucoup de précautions pour étudier les relations doses-effets. Ces test informatisés sont de plus en plus utilisés pour étudier la neurotoxicité des solvants au niveau du système nerveux central.
- l'*analyse des cofacteurs interférants dans la réponse toxique de l'organisme*. Elle peut être intéressante pour évaluer la différence de réponses interindividuelles. Par exemple, pour les études concernant les toxiques agissant par stress oxydant, il peut être utile d'analyser les antioxydants au niveau sérique.

4- Les marqueurs tardifs de toxicité. Ils intéressent : les effets cliniques, réversibles ou non, les causes de décès.

Les études correspondant à ces marqueurs sont des études épidémiologiques à la recherche des relations de causalité entre les expositions et les effets, en particulier des études de cohortes (exposés/non exposés ou cas/témoins, enquêtes de mortalité).

L'éventail des investigations concernant la surveillance des individus ou des groupes exposés est très large. Mais avant d'utiliser un marqueur biologique pour une interprétation individuelle, il faut que ce marqueur ait été validé au niveau collectif et dans le cadre des relations doses-effets. faut aussi connaître les limites de l'interprétation de résultats obtenus, et notamment connaître toutes les interférences (effets additifs, synergiques antagonistes, d'autres molécules ou états morbides ou de prédisposition). Mais dans ce domaine, la recherche n'est pas encore très avancée. Néanmoins, il ne faut pas attendre pour les mettre en oeuvre dans le cadre des retombées toxiques dans l'environnement.

Plusieurs exemples montreront que, malgré toutes ces interférences, certains marqueurs ont révélé des corrélations indubitables chez les individus exposés par le fait de la pollution de l'environnement.

L'utilisation de ces marqueurs s'avère pleine de promesses car elle évolue rapidement avec les progrès de la recherche tant fondamentale qu'épidémiologique.

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USE OF THE BACTERIAL LUMINESCENCE ASSAY TO DELINEATE POREWATER CONTAMINATION WITH PAH IN AN ALBERTA RIVER

An abandoned wood-preserving plant near the western edge of downtown Calgary, Alberta, left creosote in the ground water, from which it is slowly releasing a mixture of PAH and pentachlorophenol (PCP) to the Bow River. Field surveys supporting an ecological risk assessment for the site used toxicity testing rather than chemical measurements to delineate the extent and severity of sediment contamination. Shallow porewater samples (30-50 cm depth) taken from numerous locations with a hand-held mini-piezometer were tested for toxicity with the bacterial luminescence (Microtox®) assay. The rapidity, relatively low cost and small sample volume requirements of the test allowed sampling at sufficient points to map the spatial extent of contamination in detail. Results showed that creosote-derived toxicity extended along the near bank from about 300 m above the source area, to at least 1 km below it, but near the source area it reached to mid-channel, and in one small area toxicity was observed across the entire 100 m width of the river. The contaminated area as defined by toxicity was larger than that detected by the presence of hydrocarbon sheen, but smaller than by volatile hydrocarbon odours. The spatial survey with the bacterial luminescence assay was then used to select key sites for more intensive toxicity testing and chemical characterization of the contaminants. This use of the bacterial luminescence assay appears to hold considerable promise in assessment of aquatic and terrestrial contaminated sites.

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ASSIMILATION AND DEPURATION OF METHYLMERCURY BY CRAYFISH, ORCONECTES VIRILIS

Due to the close association of crayfish to sediments, their signifiance as a trophic link in aquatic systems and their importance as a food source for a number of fish species, fish-eating birds and piscivorous wildlife, crayfish could be significantly aiding the flux of Hg from sediments to fish and wildlife. To help understand the role that crayfish may have in mediating mercury flux, Hg uptake and depuration rates are required. A laboratory study was designed to determine 1) assimilation efficiency of $CH_3{}^{203}HgCl$ from food by O. *virilis*, 2) biological half-life of assimilated $CH_3{}^{203}HgCl$, and 3) effect of crayfish size and sex on $CH_3{}^{203}HgCl$ clearance. Mean assimilation efficiency of $CH_3{}^{203}Hg$ from tadpoles was 96 ± 0.3 % (± SE). There was no apparent difference in assimilation efficiency between size class or between sex within each size class. For the majority of the 50 experimental crayfish, no significant loss of assimilated $CH_3{}^{203}Hg$ was evident over the 166 day monitoring period. When a significant loss of $CH_3{}^{203}Hg$ was detected, observed half-lives usually fell within 1000 to 2000 days (3 to 5 years). These results suggest that crayfish could be very significant in terms of Hg dynamics in aquatic systems.

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APPORTS RÉPÉTÉS DE LISIERS ET PROPRIÉTÉS CHIMIQUES D'UN SOL LOAM ARGILEUX NEUBOIS

Au cours d'une expérience de longue durée (1978-1992), une étude a été effectuée sur les effets de l'application répétée du lisier de porc sur l'extractibilité d'éléments chimiques et sur la qualité de la solution du sol. Des échantillons de sol ont été prélevés en 1992 par couche de 20 cm jusqu'à une profondeur de 1 m. Le lisier était incorporé par aspersion en bandes à un sol loam argileux de la série Neubois (gleysol humique). Le maïs ensilage (*Zea mays L.*) a été cultivé en semis direct et sans apport minéral. Le pH du sol n'a pas été affecté de façon significative. Par contre des effets très significatifs ($P<0.01$) ont été observés pour la concentration en P extractible (Mehlich-3P), jusqu'à 60 cm de profondeur. Des effets analogues ont été notés pour l'N et le P totaux ($P<0.01$) jusqu'à une profondeur de 1 m. Les traitements ont aussi affecté tant en surface qu'en profondeur, les concentrations en C organique, la CEC, les bases échangeables, le calcium, le magnésium et le potassium extractibles ($P<0.01$) et des cations métalliques tels que le fer, l'aluminium et le manganèse échangeables ($P<0.01$). A part le pH, des paramètres de la qualité de la solution du sol tels que la conductivité électrique, la force ionique et l'indice d'adsorption du sodium ont été significativement influencées ($P<0.01$) par les traitements. Une augmentation du phosphore extractible, du potassium et du magnésium extractibles a été particulièrement notée pour les parcelles traitées avec les plus fortes doses de lisier de porc (90 et 120 M gha^{-1}). L'application de fortes doses de lisier de porc a donc augmenté la fertilité et amélioré la qualité du sol grâce à l'accroissement des teneurs en éléments extractibles. Cependant, l'accumulation à la surface du sol de certains éléments nutritifs potentiellement nocifs et leur migration possible dans le profil du sol peuvent affecter la qualité de la nappe d'eau souterraine et celle des eaux de surface.

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JOHANSSON[1] K.

MERCURY IN SWEDISH FOREST SOILS AND WATERS - ASSESSMENT OF CRITICAL LOAD

As a result of air pollution, the content of Hg in fish has significantly increased in a large part of Scandinavia and North America. In the paper, the occurrence and fluxes of Hg in Swedish forest soils and waters are reviewed and synthesized. The main objective is to describe and evaluate the present transport of anthropogenic Hg from atmospheric deposition, through the terrestrial compartment and running waters to lake basins, and also to comprehend the main factors influencing these fluxes. The transportation and distribution of Hg in forest soils and waters is closely related to the flow of organic matter. The content of Hg in humic matter is higher in southern and central areas compared to the north of the country. Compared to background concentrations, the Hg content has increased in the southern and central part by about a factor of 4-7, while the overall increase in the north is by about a factor of 2 to 3. The increased content of Hg in forest soils may have an effect on organisms and biological processes in the soil. Regarding budget calculations for whole catchment areas and for the mor layer of the soil, a reduction of about 80 % from present atmospheric wet deposition must be obtained to reach «critical load» with respect to conditions in Scandinavia.

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JONES[1] S.

KEY ISSUES IN THE ECOLOGICAL ASSESSMENT OF CHLOROETHANES

The most common approach for determining if a substance may have harmful effects on aquatic and terrestrial biota is to compare concentrations in the environment with effects thresholds determined in the laboratory or observed in the field. In addition, issues such as how the substance enters the environment and its partitioning behaviour and persistence in the environment must be considered. In Canada, chloroethanes are emitted primarily to air but are also released to surface waters and groundwater. These volatile substances partition from surface waters to air and, in the case of 1,1,1-trichloroethane, migrate to the stratosphere where it contributes to the depletion of the ozone layer. Also, the even distribution of 1,2-dichloroethane and 1,1,2,2-tetrachloroethane in Canadian ambient air suggests that they enter by long-range atmospheric transport from other countries since use and production of these substances in Canada are limited. In groundwater, chloroethanes are persistent. Based on this information, we determined three routes by which Canadian aquatic biota may be affected by chlorinated ethanes : 1) exposure to contaminated surface waters; 2) exposure to contaminated groundwater in recharge zones; and 3) exposure to elevated levels of UV-B radiation as a result of substance-mediated stratospheric ozone depletion. Each of these routes is discussed for 1,2-dichloroethane, 1,1,1-trichloroethane and 1,1,2,2-tetrachloroethane.

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BIOCONCENTRATION FROM WATERS AND BIO-ACCUMULATION FROM FOOD OF PCB CONGENERS IN RAINBOW TROUT (ONCORYNCHUS MYKISS) SUSPENDED IN CAGES IN THE ST.LAWRENCE RIVER FOR 30 DAYS

To investigate for the Mohawk Nation the potential for carrying out aquaculture in the St.Lawrence river, which is polluted by PCB, rainbow trout (30 cm) raised in a clean aquaculture environment, were confined in cages suspended in the water column at one uncontaminated and two contaminated sites in the river near Cornwall, Ontario. They were hand-fed Purina Fish Chow daily until satisfied. Fish (5) were removed from each cage after 10, 20 and 30 days. The edible filets were analysed for 68 PCB congeners, Mirex, p,p'-DDE and hexachlorobenzene by gas chromatography with electron capture detection.

At the most contaminated site (500 ng/L) equilibration was reached between 10 and 20 days. A pattern of PCB congeners resembling Aroclor 1248 but skewed somewhat toward the less chlorinated congeners, resembling the pattern of PCB in the water, was observed. Bioconcentration factors showed an unexpected trend, with lipophilic congeners showing lower factors than the more water soluble congeners. At the control site, levels were so low (30 ng/L) that, with the exception of 2-chlorobiphenyl and 2,2'-dichlorobiphenyl, the only discernable uptake showed a PCB pattern similar to the trace PCB impurity in the chow (40 ng/g total PCB). The bioaccumulation factors followed the trend expected, with the most lipophillic congeners showing the highest uptake factors.

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USE OF TRITURATE FROM MACROPHYTE SETTLED PARTICULATES FOR CONGENER SPECIFIC BIOMONITORING OF POLYCHLORINATED BIPHENYL (PCB) LEACHATE

Accumulation of PCBs within aquatic ecosystems is affected by uptake to biota, sorption to particulates, and physical redistribution throughout the water column. The site contaminated by General Motor's PCB landfill on the St.Lawrence River near Massena, NY, was studied to establish the most economical leachate monitoring technique. This investigation also determined the relative partitioning of individual PCB congeners among primary producers, abiotic substrates and higher trophic levels. Sessile plants accurately distinguish between other pollution sources to ecosystems by concentrating only localized contaminants. Integration over time is another advantage over transient biota such as fish, which require more extensive and costly sampling and analytical techniques. With aqueous samples traditionally low in hydrophobic contaminants, macrophytes were exploited to enrich the most accessible fraction without losing analytical sensitivity. Trituration extracts both settled particulates and macrophytes, generating an aqueous matrix for contaminant analysis. Total PCB levels detected in triturates ranged from 64 ng/L to 7.7 μg/L. These levels are far above minimum detection limits, which approach 1 ng/L for each congener. The use of the easily extracted aqueous matrix allows simple, economic detection of the primary entry and accumulation of xenobiotics into the food chain.

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ENVIRONMENTAL FATE AND EFFECTS OF BLEACHED PULP MILL EFFLUENTS : FOLLOW-UP STUDIES ON DIOXIN, EROD INDUCTION, AND THE ROLE OF DIETARY PATHWAYS

As part of a multi-year (1990-92) chemical and biological assessment of fish populations exposed to bleached kraft mill effluent near Grande Prairie in northern Alberta, we followed responses in the river ecosystem to changes in mill process (e.g. shift from molecular chlorine to chlorine dioxide). Although no population-level impacts were observed, several endpoints were analyzed further : dioxin/furan body burdens in the mountain whitefish *Prosopium williamsoni*; hepatic cytochrome P4501A ("MFO") in this species; and the role diet may play in uptake of P4501A inducer(s). Dioxin levels have declined steadily since replacement of molecular chlorine; data from 1993 will be reported. P4501A protein and associated enzyme activity (EROD) in whitefish collected near the mill discharge declined significantly from spring 1991 to spring 1992, with further declines by spring 1993. Impacts of a scheduled maintenance shutdown on EROD values will be discussed in light of hypotheses on fish uptake and depuration of inducers. To examine the route of uptake of inducing compounds, P4501A in intestinal samples was visualized by enhanced chemiluminescence, and P4501A cellular localization in gill, intestinal, and liver tissues was determined by immunohistochemistry. These results will be compared with dioxin body burdens. Potential implications for environmental monitoring and regulation of pulping effluents will be discussed.

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GENETICS AND APPLICATIONS OF THE TOD SYSTEM

The *tod* system refers to the toluene degradation pathway by *Pseudomonas putida* F1. A number of aromatic compounds including biphenyl and naphthalene are substrates for the toluene dioxygenase, a key enzyme complex in the *tod* pathway. Trichloroethylene (TCE), a prevalent groundwater contaminant, is also a substrate. The entire gene cluster in the *tod* pathway is now known. The cloning of *tod* genes has facilitated the expansion of the substrate range when combined with genes from other degradative pathways. Recently, we have found a novel set of regulatory genes for aromatic metabolism. These genes are a valuable new source of DNA probes for biomonitoring purposes. Genetic strategies towards the relief of toxic intermediate accumulations in degradative bacteria will be discussed.

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DISTRIBUTION AND RELATIVE TOXICITY OF PCDD's, PCDF's AND PLANAR PCB's IN THE LAURENTIAN TROUGH SEDIMENTS

Vertical distributions of polychlorinated dibenzo-*p*-dioxins (PCDD's), dibenzofurans (PCDF's) and planar biphenyls (PCB's) were determined in two sediment cores from the Laurentian trough, the principal site of fine particles deposition in the Lower St.Lawrence Estuary. Sedimentation rate, bioturbation coefficient and thickness of bioturbation were also obtained from unsupported ^{210}Pb profiles, evaluated with a steady state two-layer advection-diffusion model. Despite bioturbation activity up to a 20 cm depth in both cores, profiles of organic contaminants are characterized by a sub-surface maximum at around 10 cm below the surface. The peak concentration of total-PCDD's, total-PCDF's and planar PCB's (Σ77, 126 and 169) reaches 1 100 pg.g^{-1}, 300 pg.g^{-1} and 600 pg.g^{-1}, respectively. Octachlorodibenzo-*p*-dioxin contributes to more than 50 % of total-dioxin while 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the most toxic dioxin, is below detection limit (< 0.5 pg.g^{-1}). Octachlorodibenzofuran and 1,2,3,4,6,7,8-heptachlorodibenzofuran predominates in the group of PCDF's. The most toxic furan, 2,3,7,8-tetrachlorodibenzofuran, is always present at low concentration (1-10 pg.g^{-1}) in our samples. Among the three planar PCB's measured, 3,3',4,4'- tetrachlorobiphenyl (PCB 77) is the most abundant. The contribution of the different groups of compounds to the toxicity of Laurentian Trough sediments and the impact of bioturbation on the exposure of benthic organisms to contaminants are discussed.

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DEVELOPMENT OF ENVIRONMENTAL IMPACT ASSESSMENT TESTS BASED ON THE EXOENZYME ACTIVITY OF INDIGENOUS BACTERIA

Microbial processes are responsible for most chemical transformations in nature, including the degradation of organic compounds, the regeneration of nutrients and the transformation of trace metals. However, until recently, environmental impact studies have largely ignored the effects of contaminants on the microbiota.

The vital importance of enzymes at both the cellular and ecosystem levels is universally recognized. In bacteria for example, high molecular weight compounds must be cleaved outside the cell wall by exoenzymes before uptake can occur. Since the toxicity of most pollutants is attributed to enzyme inhibition, bioassays based on enzyme activity may be more sensitive than population level (LD_{50}) tests, and more rapid, cost-effective and reliable than measurements of whole organism activity. In recognition of this fact, experiments have been conducted to establish a standard protocol for the measurement of exoenzyme activity in marine sediments using Methylumbelliferone (MUF) and methylcoumarinylamine (MCA) based fluorogenic substrates. MUF-β-glucoside, MUF-phosphate, MCA-leucine and MCA-alanine were found to possess high activity rates within marine sediments. The sensitivity of the microbial exoenzyme systems to contaminants (e.g. water-soluble fraction of Scotian Shelf condensate) was assessed in the laboratory using experimental «aquaria-type» enclosures. Anticipated applications for this biochemical procedure include its use as a tool to monitor the immediate and long-term impacts of offshore-drilling and dredging operations on the benthic environment.

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OIL SPILL BIOREMEDIATION STUDIES IN LOW-ENERGY SHORELINE ENVIRONMENTS

Nutrient-enhanced bioremediation, a procedure by which the rate of biodegradation of pollutants is enhanced by providing indigenous microorganisms with the «limiting» nutrient, is recognized as one of the most promising of the emerging technologies for the cleanup of oil-contaminated shorelines. As a result, both government and industry have focused their attention on the commercial development and evaluation of bioremediation products.

In situ sediment enclosures have been used in our research program to test the efficacy of selected nutrient formulations to enhance the biodegradation of crude oils and condensate in low-energy shoreline environments. In most cases, the addition of organic nutrient formulations (including commercial oleophilic products) and water-soluble inorganic fertilizers stimulated microbial activity and prolonged the period of oil degradation. Time-series chemical analyses (GC-FID and GC-MS) also indicated an increase in the depletion rates of normal alkanes, and polyaromatic hydrocarbons (including toxic/carcinogenic fractions) in the residual oil following nutrient treatment.

Experimental results suggest that nutrient-enhanced bioremediation should be considered as one of the many oil spill countermeasure technologies, including the option of no treatment. However, before it becomes a practical oil spill response technology, further research and development programs are required to identify its operational limitations (under what conditions does it work) and to improve application methodologies (type of fertilizer, frequency of application, etc.). The development of experimental protocols for such studies will be discussed in the presentation.

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LE DÉPISTAGE DES SUBSTANCES GÉNOTOXIQUES DANS LES MÉLANGES COMPLEXES A L'AIDE DU SOS CHROMOTEST

Les organismes vivants, y compris l'homme, subissent un stress génotoxique sans cesse croissant. Les activités industrielles et agricoles, notamment, génèrent des contaminants qui ont le potentiel d'endommager le matériel génétique. Quoiqu'il existe actuellement peu ou pas de normes environnementales visant à contrôler strictement le rejet des substances capables de porter atteinte au génome des différentes espèces, des bioessais doivent être appliqués pour : 1) évaluer le danger et ultimement les risques; 2) étudier les mécanismes d'action des molécules génoréactives; 3) sensibiliser les législateurs à ce type de pollution. Le SOS Chromotest (*Escherichia coli* PQ37) représente un outil coût/efficace pour dépister les substances qui peuvent léser l'ADN. L'essai offre une bonne concordance avec le test d'Ames qui est considéré comme le test classique de mutagénicité bactérienne. Contrairement à ce dernier, le SOS Chromotest est simple, rapide et relativement peu coûteux. De sa version originale, en passant par celle commercialisée, le SOS Chromotest fut optimisé pour analyser les mélanges complexes non concentrés. Dans le cadre du Plan d'action Saint-Laurent 1, le test fut exploité pour caractériser la toxicité des effluents industriels et autres échantillons environnementaux. Les résultats de ces travaux et les améliorations apportées au mode opératoire seront présentés.

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BIODEGRADATION OF PENTACHLOROPHENOL AND BTEX IN A LAB-SCALE FLUIDIZED BED BIOREACTOR

One particularly promising technology for the treatment of wastewater involves the use of fluidized bed bioreactors (FBB). With proper design and operation, the FBB can have a high and active cell concentration, a low wash out of biomass, a high mass transfer and a minimum clogging of support particles, and thus, generally yield high removal efficiencies and rates of contaminants.

In this study, biodegradation of pentachlorophenol (PCP) and monocyclic aromatic hydrocarbons (BTEX) was investigated in a three-phase FBB. Support particles of the reactor, a polymer, possess a large surface area, on which either a PCP or a BTEX degrading bacterial mixed culture was immobilized. These two cultures were previously obtained by enrichment from the chemicals contaminated soils and are capable of growing in the medium with inorganic salts and target contaminants as sole source of carbon. The biodegradation tests were performed using wood washing process waters contaminated with PCP and underground waters contaminated with BTEX.

The results of the tests showed the removal of PCP greater than 99 % and BTEX to their analytical detection limits with a hydraulic retention time of less than two hours. A mass balance was made to measure the concentration of contaminants in inlet and outlet waters, gas emission and bed media. From the balance study, it was shown that biodegradation is a major reaction responsible for the contaminants removal. Based upon the investigation, the fluidized bed bioreactor would be effective for the treatment of the organic compounds in wastewater.

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EFFECTS OF 10-DAY GROWTH RETARDATION ON LONG-TERM SURVIVAL, EMERGENCE, AND FECUNDITY OF CHIRONOMUS TENTANS : POSSIBLE IMPLICATIONS FOR SEDIMENT TOXICITY ASSESSMENT

Use of the 10-day sediment toxicity test with second instar *Chironomus tentans* larvae has become widely used to assess the toxicity of freshwater sediments. This study evaluated the use of larval growth as a test endpoint by examining the relationship between statistically and biologically significant reductions in growth. We examined effects of reduced first generation (F1) larval growth on survival, time to emergence, emergence success, dry weights of adults, egg mass production, and survival and growth of second generation (F2) larvae. Larval growth, in clean silica sand, was controlled by using six feeding levels (X) ranging from 0.2 - 5.9 mg dry weight Tetrafin/day.

Larval growth (Y) was related to feeding level as described by the equation : $Y = -0.158 + 0.199X$, $r^2 = 0.99$. When 10-d larval growth was reduced by > 86 %, no adult emergence was observed although 10-d survival was > 87 %. When 10-d larval growth (F1) was reduced by 50 %, adult emergence was reduced by 85 %, mean time to emergence of males was increased by 10 d, and egg mass production reduced by > 85 % relative to observations at the recommended feeding level of ≈ 4.0 mg Tetrafin/day (controls). F2 larvae from adults fed < 4.0 mg/day grew comparably to control larvae when fed 4.0 mg/day in new 10-d tests.

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EFFECTS OF DIFLUBENZURON ON NON-TARGET INVERTEBRATES IN LITTORAL ENCLOSURES

The non-target biological effects of the insect growth regulator diflubenzuron (Dimilin) were investigated in a set of 12 littoral enclosures (10 m long X 5 m wide X 0-1.5 m deep) in northern Minnesota. Dimilin 25 W was applied on July 9, 1992 to the surface of 8 of the 12 enclosures at nominal rates of 0.7, 2.5, 7.0, and 30 µg/L (2 replicates each). Four enclosures served as controls. Abiotic and biotic parameters were sampled both before and after treatment including zooplankton, macroinvertebrates, and emergent insects.

The taxonomic groups most sensitive to Dimilin were crustacean zooplankton, especially Cladocera and immature Copepoda, and emergent insects (mostly Chironomidae). Most benthic macroinvertebrates were relatively tolerant. Cladoceran abundance was significantly reduced ($p<0.05$) at all treatment levels, with recovery occurring at 0.7 and 2.5 µg/L after 28 d. Insect emergence was similarly affected at all treatment concentrations, with emergence completely inhibited at 30 µg/L. Again, recovery was observed at 0.7 and 2.5 µg/L by day 28. Recovery at the two highest treatments was poor for most crustacean and insect populations.

It was concluded that single surface applications with Dimilin resulted in minor and transient, but statistically significant, reductions in sensitive aquatic invertebrate taxa, even after treatment with the lowest test concentration, 0.7 µg/L. Impact was progressively greater, and duration of effects longer, at higher Dimilin concentrations.

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USE OF WILDLIFE MODELS IN THE ASSESSMENT OF PRIORITY SUBSTANCES IN CANADA

Toxicity to wildlife (birds, mammals, amphibians and reptiles) and their habitat is assessed as part of the determination of «toxic» under the *Canadian Environmental Protection Act.* For each priority substance, estimates of inhalation and oral (i.e., eating and drinking) exposure are determined for a specific model wildlife species. The species chosen depends on the substance, the location and environment (aquatic, terrestrial) of estimated highest exposure and the primary route of exposure. Estimates of exposure are compared to available toxicological data, usually no-observed-effect levels from chronic laboratory studies, although field studies are preferably selected if available. When necessary, various assessment factors are used to account for extrapolations, such as from laboratory to field situations, and subchronic to chronic endpoints. Several case studies will be presented to illustrate this method of assessment of toxicity to wildlife.

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INDICATIONS OF INCREASED MERCURY INPUTS TO NORTHERN CANADIAN LAKES AS A RESULT OF ATMOSPHERIC FALLOUT

Since the late 1960s there has been continuous research on the environmental occurrence of mercury in Canada. Early research focussed on sites of electrolytic production of chlorine using elemental mercury as a cathode. More recent research on mercury levels in people has shown increased frequencies of elevated levels in people living in coastal arctic communities. These communities do not have local industrial activities and they do not occupy sites rich in mercury-containing minerals. The main source of mercury to these people is their consumption of arctic animals, notably marine mammals and freshwater fish. Studies of hydroelectric reservoirs have shown that mercury in fish increases consistently following impoundment. This increase, driven by the increased availability of organic carbon which favours methylation of mercury over demethylation, last varying lengths of time. For fish feeding at high trophic levels, the phenomenon lasts longer than for fish feeding at low trophic levels. Indeed in the former case, the duration of the increase remains largely unknown. More recent studies of mercury in air and in lake sediment cores have detected increased inputs of mercury over a broad geographic area, apparently as a result of atmospheric inputs. Studies of gaseous mercury in air over the Atlantic Ocean have indicated that concentrations are increasing at a rate of 1.46 per cent per year, indicating a doubling time of less than half a century. Studies of lake sediment cores along a north/south transect in central Canada have also suggested striking recent increases in mercury. It is not known whether the increased inputs of mercury result in increased biological cycling of this element. However, in some instances there are statistical associations between high mercury inputs and high levels in fish. If these associations prove to be reliable, then the trend is ominous for people who regularly consume northern fish. Northern ecosystems are faced with three types of inputs, namely natural geological background levels, inputs as a result of local human activities, and fallout originating from distant natural or human activity. Our ability to control mercury inputs in the North will likely depend on our ability to partition total amounts found among these three sources since each will require a different control strategy.

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POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS OF ISOLATED NORTHERN CANADIAN LAKES

Sediment cores were taken from several northern Canadian lakes along a transect from the Experimental Lakes Area (latitude 49° N) to northern Ellesmere Island (latitude 82° N). Cores were sliced at collection sites and individual slices were returned to the Freshwater Institute for analysis. Sediment in each slice was weighed and freeze-dried prior to radiochemical determinations of lead-210 and cesium-137 for dating purposes. Slice material was then soxhlet extracted with dichloromethane, cleaned up with silica gel, and analyzed for a number of polycyclic aromatic hydrocarbons (PAHs) by GC/MSD (SIM) using several deuterated PAHs as internal standards. Different individual PAHs showed quite different patterns in the same core. The lighter, more water-soluble PAHs tended to show little trend with core depth while the heavier four and five-ring compounds usually were enriched in the top slices relative to deeper ones. An exception to this was perylene which typically increased with depth in the core, presumably because of *in situ* formation there. Considering the sum of PAHs, the pattern at the Experimental Lakes Area was one of a low background followed by a striking increase during the first half of this century, and then a recent decline, although not to historical background levels. The same pattern was evident at the next site, Hawk Lake near the west coast of Hudson Bay, except that all the values were lower. Further north, on Cornwallis Island the pattern showed only a very modest (about 2-fold) increase in current levels relative to deeper ones. The most northerly site, Hazen Lake on northern Ellesmere Island, showed a very unusual pattern; the PAHs decreased consistently with depth. The watershed of this lake is rich in coal, and the core material contained many bits of unburned coal and amber. Samples of both coal and amber were collected and analyzed and showed high concentrations of perylene and retene. Probably the unusual PAH profile from lake Hazen reflected erosion of coal rather than long-range transport. It appears that high latitude lakes are receiving low but continuing inputs of PAHs as a result of fallout. Both basal levels and current levels are quite sensitive to latitude. The biological meaning, if any, of these inputs is problematic.

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MÉTAUX TRACES DANS LES SÉDIMENTS DU SAINT-LAURENT

Depuis 1989, plusieurs études portant sur la caractérisation de la contamination des sédiments de surface et sur la chronologie de la pollution par les métaux traces ont été réalisées dans le tronçon Cornwall à Trois-Rivières. Les différents sites échantillonnés présentent des profils représentatifs des sources locales, mais dont le signal se superpose à celui des Grands Lacs et des autres sources de l'amont. De plus, pour de courtes périodes la relation significative entre les concentrations et le débit indique ce dernier a une influence sur les concentrations de métaux traces. A long terme, l'augmentation significative du pH moyen observée à plusieurs sites depuis les 50 à 100 dernières années, pourrait en partie expliquer l'augmentation synchrone des concentrations de certains métaux traces. Malgré cela, certaines observations générales son possibles. La contamination par le Ni et le Cr n'est pas un problème dans le tronçon étudié pour les périodes de temps considérées, alors que le facteur d'enrichissement anthropique (FEA) est proche de l'unité. Dans le cas du Pb, la diminution de la concentration est observée depuis la fin des années 1960 ou depuis le début des années 1980 selon les sites. A l'aval de la zone d'étude, le FEA pour Cd, Cu et Zn semble avoir augmenté, alors qu'à l'amont il est resté relativement constant depuis les 20 ou 30 dernières années. Finalement, l'apport en métaux traces provenant des Grands Lacs étaient beaucoup plus important que les sources locales dans les années 1960, en particulier pour le Cd et le Zn, alors qu'aujourd'hui le FEA des sédiments de surface est comparable.

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SOURCES AND FATE OF MERCURY IN HYDROELECTRIC RESERVOIRS OF NORTHERN QUEBEC

Since its inception four years ago, the multi-sponsored collaborative project on mercury biogeochemistry in northern reservoirs has made great strides, both in terms of analytical capabilities and conceptually. The multitude of abiotic and biotic topics investigated to date by the research team now allow us to propose a first comprehensive description of the biogeochemical behaviour of this heavy metal in reservoir systems.

Most of the airborne mercury of anthropogenic origin falling to the ground is retained by the organic matter of the forest soils. After impoundment, microbial degradation of the labile organic fraction leads to strongly reducing conditions, methane and carbon dioxide evasion, and methylation of 10 to 30 % of the Hg initially present in the flooded soils. Quantitatively, however, net loss of Hg or carbon is undetectable in the flooded soils. Furthermore, measured Hg fluxes across the sediment-water interface are too small to account for the rapid and massive contamination of the biota in the reservoirs. Other processes such as wave and ice erosion in the drawdown zone, emergence of burrowing insect larvae, and grazing of the periphytonic film appear to be responsible for actively transferring Hg from the flooded soils to the water column and its biota. The team is presently working at quantifying the role of these various key processes on the dynamics of Hg in the reservoirs.

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DÉVELOPPEMENT DES MÉTHODES POUR LA DÉTERMINATION DU MÉTHYLMERCURE, A DES NIVEAUX DE TRACES, DANS DIVERS ÉCHANTILLONS ENVIRONNEMENTAUX

Les méthodes décrites consistent essentiellement en une séparation par chromatographie en phase gazeuse et par détection spécifique du mercure, basée sur la spectrophotométrie de fluorescence atomique à vapeur froide. La faible concentration de méthylmercure dans les échantillons d'eau est déterminée après la réaction d'éthylation qui permet de convertir le CH_3Hg^+ (peu volatil) en $CH_3HgC_2H_5$ (très volatil) qui est ensuite purgé de la solution et trappé sur une colonne d'adsorbant. Le composé éthylé est ensuite désorbé thermiquement et injecté dans le GC. En ce qui concerne l'échantillon de sédiment, le CH_3Hg^+ est décomplexé de sa matrice par un mélange de : $CuSO_4$-H_2SO_4-KBr et extrait dans le toluène. L'extrait est préconcentré par évaporation du solvant et injecté dans le GC pour analyse. Pour la chair de poisson, le traitement comporte les étapes suivantes : homogénéisation, dégraissage et clivage du méthylmercure lié aux protéines avec HCl 6N. L'analyse est effectuée par GC après extraction dans le toluène. Pour les micro-analyses (1 à 10 mg), l'utilisation de standard interne (chlorure d'éthylmercure) s'avère essentielle. La quantité minimale détectable est de 0.2pg de CH_3HgCl. Les échantillons de matières particulaires, de périphyton et d'insectes nécessitent une distillation préalable pour éviter les interférences. Les limites de détection obtenues sont : 0.02 pg/ml pour l'échantillon d'eau, 0.02 ng/g pour les échantillons de chair de poisson ou de sédiment. La précision de la technique est de l'ordre de ± 5 %.

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THE CEPA PRIORITY SUBSTANCES ASSESSMENT PROGRAM

The Canadian Environmental Protection Act (CEPA) requires the federal government, in part, to conduct ecological assessments on priority substances to determine whether they cause or may cause harmful effects to the environment. Ecological assessments have been completed for 44 substances that included a variety of volatile organic compounds (e.g., benzene, toluene), organochlorines (e.g., trichloroethylene, hexachlorobenzene), metals (e.g., arsenic, chromium), mixtures (e.g., non-pesticidal organotins, polycyclic aromatic hydrocarbons), and effluents (e.g., chlorinated wastewater effluents). This presentation will describe the Priority Substances Assessment Programe and its objectives, procedures and products. Furthermore, current linkages with the scientific community will be described, and possible ways to enhance these linkages will be discussed.

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ASSESSING THE AQUATIC TOXICITY OF MIXTURES : CAN TOXICANT BODY RESIDUES ADVANCE THE STATE OF THE ART?

The current status of residue-based toxicity interpretation in aquatic systems has recently been reviewed by McCarty and Mackay (1993). Several conclusions can be drawn :

1. Ranges of body residues can be associated with different modes of toxic action
2. Ranges of body residues can be associated with various acute and chronic bioassay endpoints
3. Exposure-based estimates of dose are often much less reliable and subject to more confounding factors than organism-based dose estimates.

These conclusions have substantial significance with respect to understanding mixture toxicity. The current paradigm for investigating and interpreting mixture toxicity is still primarily the toxic unit concept which came into use in environmental toxicity over 30 years ago. This system is basically a system for categorizing whether the toxicity of a mixture appears to be additive, synergistic, antagonistic, or not interactive at all. It offers little in the way of a scientific explanation of why mixtures are placed in the same category.

Much of the problem is not with the toxic unit concept itself, rather it is largely a limitation of the bioassay data employed. The bulk of the work that has been done with this system has employed exposure-based toxicity data. The advantages and benefits of using residue-based toxicity information in the basic toxic unit approach to investigating mixture toxicity will be examined.

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EVALUATION OF THE STEROID BIOSYNTHETIC CAPACITY OF OVARIAN FOLLICLES FROM WHITE SUCKER EXPOSED TO BLEACHED KRAFT MILL EFFLUENT (BKME)

White sucker exposed to BKME have reduced circulating levels of gonadal sex steroids which correlate with other reproductive impacts including reduced gonadal size, delayed maturity and reduced expression of secondary sexual characteristics. Previous studies have shown that BKME exposure affects reproduction by acting at multiple sites within the pituitary-gonadal axis. One site of impact is at the level of steroid production within the ovarian follicle. BKME effects on ovarian function however, are selective and do not reflect a general impairment of ovarian function. The present studies were conducted to evaluate the sites within the steroid biosynthetic pathway that are affected by BKME exposure. Specifically, an *in vitro* egg incubation procedure was developed which permits the determination of whether the reduced steroid synthetic capacity reflects a reduced availability of precursor substrates, such as cholesterol, or an inhibition or shift in the regulatory enzymes controlling steroid biosynthesis. Follicles of fish exposed to BKME show a reduced capacity to produce the major biologically active steroids (17β-estradiol, testosterone, and 17α, 20β-dihydroxy-4-pregnen-3-one) under basal and human chorionic gonadotropin stimulated conditions. Multiple sites of disruption within the biosynthetic pathway are responsible for the reduced overall steroid production and these disruptions change with the reproductive status of the fish.

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METABOLISM AND SUBTLE TOXIC EFFECTS OF BUTYLTIN COMPOUNDS IN STARFISH

The starfish *Leptasterias polaris*, an important benthic predator in the North Eastern Atlantic coast, was used to assess the potential degradation and the toxicological implications of tributyltin (TBT) ingested through the diet. TBT was chosen as a representative of the organotin compounds, a group of wide-spread biocides and plastic stabilizers. Individual starfish were maintained in running sea water and fed daily with TBT contaminated mussels. The TBT burden remained essentially constant in the digestive system throughout the 53-d experiment. Degradation metabolites were detected in the pyloric caeca with dibutyltin (DBT) appearing in the first weeks, and monobutyltin (MBT) only traced in few individuals after 6 weeks. Less than 20 % of the total tin recovered in the organs was detected as butyltins, suggesting a debutylation pathway to inorganic tin and/or to undetected organic species. Although the contaminant remained barely detectable in the gonads, a histological examination revealed significantly smaller oocytes and thinner reserves in gonadal epithelia of the contaminated individuals. These results show that starfish, and possibly other echinoderms, avoid immediate accumulation of TBT from food by dealkylating the toxicant into the less harmful metabolites, DBT and MBT. However, this natural defence process was only partly effective and effects on reproductive organs were induced by disturbing reserve acquisition in growing gametes.

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METCALFE[1] C.D.

BIOMARKERS IN ECOSYSTEM HEALTH MONITORING

Various biochemical, physiological and pathological parameters in terrestrial and aquatic organisms have been used as «biomarkers» of the health of ecosystems. However, while protection of «ecosystem health» is often the stated aim of these studies, the real reason for monitoring the health of organisms is often to protect human health or to conserve populations of organisms judged by man to have «value». An understanding of the motivations behind studies of ecosystem health is necessary to understand the resistance to biomarker monitoring within the regulatory community. High prevalences of liver tumours have been reported in several populations of bottom-dwelling fish from industrialized regions of North America. Various biomarkers of fish health, including histologically-visible hepatic lesions, elevated hepatic monooxygenase activity, fluorescent bile metabolites, aromatic DNA adducts and elevated numbers of hepatic micronuclei have clearly linked the development of liver tumours in fish with exposure to carcinogenic aromatic compounds in bottom sediments, and have established these parameters as excellent biomarkers for monitoring the health of aquatic ecosystems. However, the presentation of these data to the regulatory community is often greeted with the question, «So what?» In order to entrench the concept of biomarkers as ecosystem monitoring tools, there are some clear research needs. In addition, there is a need to clearly identify the motivations behind these studies and to decide whether protection of «ecosystem health» is the regulatory goal.

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MOLLER[1] A., MURPHY[1] T., THACHUK[1] J., BROUWER[2] H.

SULFIDE TOXICITY IN ANOXIC SEDIMENTS

In Hamilton Harbour, sulfides and reduced oxygen (redox) are high in sediments with high coal tar concentrations and decrease as the coal tar concentrations decrease.

A seasonal study of sediment from Stelco boatslip, Hamilton Harbour, showed that PAH contaminated sediment which is acutely toxic when anoxic (summer) is not toxic during the winter months when it is oxygenated (<10 % mortality). When the same oxygenated sediment was forced to anoxia, it became toxic (98 % mortality). In oxic conditions, the sulfides in the sediment are bound and not bioavailable. However, under anoxia, sulfur is reduced, thus causing toxicity.

Organisms have different exposure levels to reduced sulfur. *Photobacterium phosphoreum*, in a direct contact bioassay shows a positive correlation between acute toxicity (99 % inhibition) and sulfide concentrations (HVS : 32 ppm, AVS: 173 ppm) in the sediments. The ATP-TOX and Toxi-Chromotest bioassays, which use sediment extracts, did not show a correlation. When sediment originally non-toxic in these bioassays (34 %, 22 % inhibition) (HVS : 10 ppm, AVS: 92 ppm) was spiked with H_2S (HVS: 147 ppm, AVS:404 ppm), it was not immediately toxic to the organisms. However, when examined 22 days later, the sediment increased in toxicity (46 %, 84 % inhibition) even though the reduced sulfur concentrations had dropped (HVS: 19 ppm, AVS: 146 ppm). The hydrogen sulfide caused toxicity to these organisms indirectly by changing the sediment chemistry.

A study of the surface (0-1 cm) sediments throughout the harbour showed little toxicity and low concentrations of reduced sulfur.

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MONITA[1] D.M.A., DAVIES[1] R.W.

THE ECOLOGICAL SIGNIFICANCE OF AN ACUTE AND A CHRONIC TOXICITY BIOASSAY

The results of an acute toxicity bioassay were compared to the results of a chronic toxicity bioassay. Although the acute toxicity bioassay is a relatively rapid and inexpensive means of evaluating the effects of a stress to an organism, it provides the investigator with little ecologically meaningful information. By monitoring how energy is allocated to various physiological components during and after a stress event, an investigator could see how correlates of fitness are affected by the stress event. The acute toxicity bioassay was performed in accordance to standardized federal procedures (for rainbow trout). The results were compared to results obtained using the bioenergetic approach to chronic toxicity assessment. Acute toxicity bioassays provided the investigator only with the death of the animal exposed to a stressor, whereas the bioenergetic approach provided information on changes in growth rate, fecundity, maintenance respiration (used to combat the effects of the stress, and for tissue repair), time spent in activity, and energy allocated to storage (for use in reproduction). The bioenergetic approach to chronic toxicity assessment provided ecologically significant information upon which management decisions could be made. Methods for making the bioenergetic approach both rapid and inexpensive will be discussed. The ecological significance of the results of the acute toxicity bioassay are questioned.

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MONITA[1] D.M.A., GRAY[1] H.M., DAVIES[1] R.W.

A BIOENERGETIC APPROACH TO CHRONIC TOXICITY ASSESSMENT

Acute lethality and chronic toxicity tests have been standardized in Canadian regulations to monitor the potential effects of toxicants in aquatic ecosystems. The tests have received considerable criticism for their use of non-indigenous species and the lack of information they provide on long-term potential ramifications of the toxic substance to the aquatic ecosystem. A bioenergetic approach is proposed as a rapid and reliable means of monitoring chronic stress in aquatic environments. The approach provides the researcher with ecologically significant information upon which appropriate management decisions can be made. An indicator species common in lotic and lentic ecosystems in Canada, was used to evaluate the potential effects of cadmium toxicity. All components of a bioenergetic model were monitored as the animal was exposed to three concentrations of cadmium. Under stressed conditions, more energy was allocated towards maintenance respiration, somatic and reproductive growth. However, as the cadmium concentration increased, time to maturity and fecundity decreased. The effects of the stress were evident after the animals were removed from the cadmium solutions and placed in non-stressed conditions. Potential population effects, and changes in community structure will be discussed. A bioenergetic approach to aquatic toxicity assessment is proposed as a rapid and comprehensive tool for assessing the effects of stress in the natural environment.

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MONTGOMERY[1] S., MUCCI[1] A., LUCOTTE[1] M., PICHET[1] P.

DISSOLVED MERCURY PROFILES IN THE WATER COLUMN AND PORE WATERS OF NATURAL AND ARTIFICIAL AQUATIC SYSTEMS OF NORTHERN QUEBEC

Dissolved mercury was determined in the water column and interstitial waters of natural lakes, in and above various types of flooded soils, and peat bogs in the vicinity of the La Grande-2 and Laforge-1 hydroelectric reservoirs in northern Quebec. Pore waters were obtained with diver-positioned peepers which were allowed to equilibrate for 6 days. Water samples were analyzed in the field laboratory within 12 hours of recovery.

Total dissolved mercury concentrations, $[Hg]_{TOT-D}$, are nearly constant in unstratified water columns and rarely exceed 3.5 ppt (15 pM). $[Hg]_{TOT-D}$ increase sharply near the sediment-water interface. In natural lakes and flooded soils from the LG-2 region (impounded 12 years ago), the shape of the pore water $[Hg]_{TOT-D}$ profile is similar to that of the total mercury distribution in the sediments, $[Hg]_{TOT-S}$. The partition coefficient of mercury between the dissolved and solid phases varies between 2-10 x 10^5. In natural peat bogs, pore water $[Hg]_{TOT-D}$ increase with depth and are closely correlated to the DOC concentration.

Despite the large positive concentration gradients observed between the water column and sediment pore water, mercury fluxes at the sediment-water interface, measured using benthic chambers installed over a 24 hour period, were slightly negative (to the sediment) in natural lakes and only slightly positive (to the water column) over flooded soils.

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MOORE[1] D.R.J., CHÉNIER[1] R., FORTIN[1] C., McBAIN[1] D.C.

CURRENT FRAMEWORK USED TO CONDUCT ECOLOGICAL ASSESSMENTS OF PRIORITY SUBSTANCES UNDER CEPA

According to the Canadian Environmental Protection Act, a substance is «toxic» to the environment if «it is entering or may enter the environment in a quantity or concentration or under conditions... having or that may have an immediate or long-term harmful effect on the environment». Examination of this definition indicates that there are three major components in a CEPA ecological assessment : (i) entry to the environment, (ii) exposure of biota in the enviroment, and (iii) effects to the environment. CEPA further states that the environment includes all living organisms, interacting natural systems, and all compartments of the environment. To establish and quantify entry to the environment, information is collected on concentrations in emissions, emission rates, imports, production, exports, domestic consumption, etc. Exposure to biota can be determined using models to predict concentrations in these media. Effects to the environment may be determined using field studies, and by comparing substance concentrations in the Canadian environment to laboratory-derived acceptable concentrations for sensitive organisms. In this presentation, existing case studies for several priority substances will be used to illustrate the CEPA ecological assessment framework.

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MORIN[1] P.E., AVERILL[1] D., DENIZEAU[1] F.

EFFET D'UN STRESS THERMIQUE SUR LA VIABILITÉ ET LE CA^{2+} LIBRE INTRACELLULAIRE CHEZ DES HÉPATOCYTES DE TRUITE ARC-EN-CIEL

L'effet d'un stress thermique sur la viabilité et la concentration de Ca^{2+} libre intracellulaire ($[Ca^{2+}]_i$) a été évalué chez des hépatocytes de truite arc-en-ciel fraîchement isolés.

A 25 et 35° C, la viabilité est stable (>90 %) pour une période de 3 h. La viabilité commence à diminuer après 60 et 50 min d'incubation à 35 et 40° C respectivement. La viabilité passe de 97,7 à 92,2 % à 35° C et de 95,6 à 80,1 % à 40° C.

Les hépatocytes de truite ont une $[Ca^{2+}]_i$ basale de 92,74 ± 13,00 nM (S.E.) telle qu'évaluée à l'aide de la sonde quin2. Après une incubation de 30 min à 35 et 40° C, la $[Ca^{2+}]_i$ augmente de façon significative jusqu'à 145,91 ± 12,96 nM et 159,82 ± 14,68 nM respectivement. Après 60 minutes d'incubation, il n'y a pas de nouvelles modifications de la $[Ca^{2+}]_i$ à 35° C, tandis qu'à 40° C elle atteint 403,82 ± 71,74 nM. Une incubation à 30° C n'a pas causé de changement significatif de la $[Ca^{2+}]_i$. La hausse de ca^{2+} provoquée par le choc thermique n'est pas immédiatement cytotoxique. Nous étudions actuellement la possibilité d'un effet cytoprotecteur des protéines de choc thermique chez des hépatocytes rendus thermotolérants.

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MORRISON[1] H.L., LAZAR[1] R., HAFFNER[1] G.D.

A LABORATORY AND FIELD BASED DETERMINATION OF THE ELIMINATION AND UPTAKE RATE CONSTANTS FOR PCBs IN DREISSENA POLYMORPHA

A series of laboratory and field based toxicokinetic experiments were performed in order to determine the elimination and uptake rates of various PCBs in *Dreissena polymorpha* Pallas. Zebra mussels containing a known amount of chemical were placed in aquaria containing «clean» water. One group of zebra mussels were fed throughout the course of the experiment while the other group were not fed. Five grams of zebra mussel tissue were removed after 0,1,2,4,8,12 and 16 days. The elimination rate constants (k_2) were calculated for fed and starved zebra mussels and a comparison was made between the two conditions. The uptake rate constants (k_1) were calculated using the k_2 values determined from the starved zebra mussels and compared with uptake rate constants determined from a field based contaminant uptake study.

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MUNGER[1] C., HARE[1] L.

RELATIVE IMPORTANCE OF WATER AND FOOD AS TRACE METAL SOURCES TO LARVAE OF THE AQUATIC INSECT CHAOBORUS (DIPTERA)

To facilitate the use of *Chaoborus* as a bioindicator and to model metal dynamics in the biota of lakes, it is incumbent to know the relative importance of water and food as metal sources for these insects. To estimate this, we are studying the accumulation of cadmium along a food chain composed of the green alga *Selenastrum capricornutum*, the herbivorous cladoceran *Ceriodaphnia dubia*, and the predatory insect *Chaoborus punctipennis*. The results of preliminary experiments suggest a rapid accumulation of cadmium by the alga and toxic effects at a cadmium free-ion concentration of 1 X 10^{-9} M. To estimate the proportion of cadmium accumulated from food and water we will measure the difference in ^{109}Cd concentrations in *Chaoborus* held either in ^{109}Cd-free or ^{109}Cd-rich water and fed cladocerans raised on either ^{109}Cd-free or ^{109}Cd-rich algae.

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MURPHY[1] T., MOLLER[1] A., THACHUK[1] J., BROUWER[2] H., BABIN[3] J., FOX[1] M, DON[1] H.

IN SITU SEDIMENT BIOREMEDIATION IN HAMILTON HARBOUR AND ST.MARYS RIVER

To enhance the biodegradation of organic contaminants, approximately 30 tonnes of oxidant were injected in 1992 into 3 hectares of sediments of Hamilton Harbour and the St.Marys River.

In Hamilton, about 80 % of the hydrogen sulphide (293 µg/L to 53 µg/L at deep basin) was oxidized. Oxidation resulted in precipitation of about 98 % of the porewater iron in the surface 15 cm of sediment but the concentrations of most trace metals were unchanged. In the Dofasco boatslip biodegradation of organic contaminants varied from 79 % for low molecular weight compounds (BTXs), to 25 % for petroleum hydrocarbons, and 15 % for polynuclear aromatic hydrocarbons. More time is required for bacteria to metabolize the higher molecular weight organic wastes.

The treatment of the St.Marys River sediments resulted in oxidation of more than 90 % of the hydrogen sulphide. However, for the first year of monitoring, the high variability in the sediment composition masked any effect of the treatment on biodegradation. Laboratory experiments indicated 95 % biodegradation of TPHs and 50 % biodegradation of PAHs within a year. Ongoing studies indicate the *in situ* biodegradation could be impeded from methane diffusion in deeper sediments. Treatments in 1993 injected oxidants deeper, released methane, and some treatments also used an organic amendment.

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NALEWAJKO[1] C.

ALGAE AS TOOLS IN AQUATIC TOXICITY TESTS

Laboratory bioassays with cultures of algae have been used since the 1950's to test potential effects on algal growth of nutrients, various contaminants, as well as complex effluents. Photosynthetic rates have also been used as the criterion of algal response, but to a lesser extent. We have explored the use of light saturated photosynthetic rates in bioassays, and have found this approach particularly useful in initial screening tests. Typically, EC50 values from growth experiments were an order of magnitude lower than those derived from photosynthesis experiments : the former are chronic and the latter acute toxicity tests.

The choice of algal species for the bioassays is important because, for some contaminants at least, large differences have been documented in responses among different algal genera, and in some cases, also among species of the same genus. Furthermore, gradual replacement of extant taxa by pollution tolerant species or genotypes in some contaminated lakes subjected to chronic pollution may invalidate laboratory bioassay results obtained with standard test organisms. The use of natural phytoplankton communities in bioassays may be preferable for this reason.

The physiological state of bioassay cultures must be standardised in order to avoid the confounding effects of nutrient status, (eg. differences in cell P, N or C metabolism) on algal responses to toxic substances. The P state of the culture is an important factor controlling the extent of metal (Cu, V) toxicity as well as the toxicity of contaminated sediments to algal photosynthesis. In species that rely on metal exclusion as the tolerance mechanism, N starvation may enhance extracelluar fibril production, and hence increase metal binding.

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NANTEL[1] A.J.

L'IMPORTANCE DE LA TOXICOLOGIE AQUATIQUE POUR LA SANTÉ HUMAINE

Pour les professionnels de la santé, le concept de toxicologie aquatique faisait surtout référence, jusqu'à tout récemment, aux pathologies humaines provoquées par divers organismes marins tels l'intoxication paralysante par les mollusques causée par le gonyaulax; les intoxications par la ciguatoxine, la scombrotoxine, la sanitoxine, la tetrodotoxine, la neurotoxine et la batratoxine causées par l'ingestion de diverses espèces de poissons, de mollusques ou de certaines algues (les algues bleues-vertes); ou les piqûres ou morsures causées par des poissons ou des serpents marins (ex. : la raie armée, la rascasse, la vive, etc.). Depuis environ deux décennies, un nouvel aspect de la toxicologie marine pouvant affecter la santé humaine s'est imposé tant dans le milieu scientifique que la perception publique. Il s'agit de la contamination de la chaîne alimentaire par le méthylmercure et les dérivés organochlorés. La situation particulière du Canada à cet égard en a fait un sujet de recherche prioritaire.

La contamination chimique des eaux de surface, des eaux souterraines et des sédiments est aussi devenue, au cours des dernières années, un sujet de préoccupation croissante. Par ailleurs, bien que préoccupés par la situation actuelle, les spécialistes de la santé ont encore beaucoup de mal à définir l'impact réel de ce type de pollution sur l'organisme humain. Il existe encore beaucoup de mal à définir l'impact réel de ce type de pollution sur l'organisme humain. Il existe encore beaucoup de confusion dans ce domaine entre l'importance relative du risque pour les espèces marines exposées par rapport aux risques pour les humains. Quoiqu'il en soit, on accepte de plus en plus le concept selon lequel l'homme fait partie intégrale de l'écosystème et que tout ce qui affecte l'air, l'eau, le sol, la flore et la faune risque, un jour, de l'affecter lui-même. Les divers sujets qui seront traités durant les prochains jours illustrent bien cette réalité.

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NANTEL[1] A.J.

IMPACT OF AQUATIC TOXICOLOGY ON HUMAN HEALTH

For health professionals, aquatic toxicology referred mainly, up to recent times, to human diseases caused by various marine organisms such as paralytic shellfish poisoning caused by gonyaulax; ciguatoxin, scombrotoxin, sanitoxin, tetrodotoxin, neurotoxin and batratoxin poisonings caused by ingestion of various species of fishes, shellfishes and some algae (like blue-green algae); or stings and bites caused by fishes and marine snakes (ex. : stingray, scorpion fish, weever, etc.). Over the past two decades, a new aspect of marine toxicology likely to affect human health has commanded attention of both the scientific community and the general public. It related to food-chain contamination by methyl-mercury and organo-chlorine derivatives. The specific Canadian situation in this regard has made it a high priority research area.

Chemical contamination of surface and groundwaters along with sediments has also become in recent years a subject of increasing concern. However, even though they are aware of the seriousness of this situation, health professionals are still unable to measure quantitatively the exact impact of this type of pollution on human health. One may still observe significant confusion regarding the relative risk that these pollutants represent for marine life and human health. Nevertheless, the general concept that man is an indissociable part of the ecosystem is gaining approval in the sense that he may one day be affected by any deterioration of the quality of air, water, soil, flora and animal life. The very diversified subjects which will be presented during the next few days illustrate this reality.

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NOTON[1] L.R., MACDONALD[2] G., MACKENZIE[3] I.B.

PULP MILL EFFLUENT AND OTHER FACTORS IN THE WINTER OXYGEN BALANCE OF THE ATHABASCA RIVER

The Athabasca River flows 1300 km from the Rocky Mountains across northern Alberta to Lake Athabasca. For 3-4- months in winter it is ice-covered, has low flow, and develops oxygen saturation deficits. Expansion in the pulp mill industry, which discharges effluent to the river, has led to increased investigation of winter oxygen dynamics. This has included synoptic sampling of the mainstem, tributaries, and effluents at river time-of-travel, continuous oxygen monitoring with recording meters, streambed oxygen demand (SOD) measurements, and reaeration rate investigations. In addition to pulp mill effluent, important factors in the winter oxygen balance are discharge, headwater and tributary oxygen inputs, extent of ice-free areas, re-aeration at Grand Rapids, streambed oxygen demand, oxygen demanding material in other effluents and tributaries, and in some winters, photosynthesis under ice. Results of investigations on these factors and general oxygen conditions will be described.

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OLAVESON[1] M.M., NALEWAJKO[1] C.

USE OF BIOASSAYS FOR ASSESSMENT OF METAL TOXICITY USING ACID MINE DRAINAGE ALGAE

Standardized algal growth bioassays with commonly-used laboratory cultures (e.g. *Chlorella, Selenastrum*) do not accurately reflect the toxicity of contaminated wastewaters to primary producers especially when testing waters with unusual characteristics (e.g. extreme pHs or temperatures). Many such contaminated wastewaters have functional communities of phototrophs and heterotrophs even though species diversity is low. The examination of the indigenous species from such wastewaters is both interesting, in an autecological sense, and potentially useful in providing a greater variety of alternative species for assessing wasterwater toxicity.

Algal species isolated from metal-contaminated waters (e.g. acid mine drainage) are particularly useful for determining the effects of various metals on growth and various physiological processes (e.g. photosynthesis, respiration). *Euglena mutabilis*, a pioneering algal species in acid mine drainage (characterized by low pH and high metal - Al, Cd, Cu, Fe, Mn, Ni, Pb, Zn- concentrations), demonstrates resistance to a variety of metals, in both long-term growth and short-term photosynthesis bioassays. However, a related species, *Euglena gracilis*, shows significantly greater sensitivity to metals in similar bioassays. Both species are acid-tolerant.

The results of metal bioassays with both species will stress the importance of algal species selection and of bioassay design. Defining the growth and physiological responses of bioassay species to target contaminants is crucial in assessing wastewater toxicity.

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O'MALLEY[1] V.P., ABRAJANO[1] T.A., HELLOU[2] J. WINSOR[1] L., STARK[1] A.

$^{13}C/^{12}C$ RATIOS OF INDIVIDUAL PAH FROM MARINE AND ESTUARINE SEDIMENTS

Anthropogenic emissions contribute the greatest proportion of polycyclic aromatic hydrocarbons (PAH) to modern estuarine environments. Because PAH are hydrophobic and lipophilic, they tend to accumulate in sediments of aquatic systems. Sediments thus offer the potential for a holistic evaluation of the relative importance of PAH contributors as a function of both space and time in aquatic ecosystems. Our approach in the evaluation of PAH sources in sediments is to utilize, in addition to molecular signatures, the variations in ratios of $^{13}C/^{12}C$ of individual PAH. First, we will describe the procedures for the collection and isolation of PAH in environmental samples for compound-specific carbon isotope analysis. Analysis of $\delta^{13}C$ of individual aromatic compounds including isomeric parental PAH can be accomplished with a precision of 0.2 to 0.3 % for well resolved peaks. Finally, the potential of compound-specific carbon isotope analysis to evaluating multiple source contributions to estuarine systems will be demonstrated using our on-going studies of selected systems in eastern Canada.

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PADRÓS[1] J., SIRON[2] R., PELLETIER[2] É., DELILLE[3] D.

EFFETS D'UN NOUVEL AGENT DE TRAITEMENT SUR LA BIODÉGRADATION DU PÉTROLE EN MILIEU MARIN

Afin de réduire l'impact des hydrocarbures sur le milieu marin en général et sur les organismes de la frange littorale en particulier, une famille de nouveaux produits anti-adhésifs a été développée. Dans le cadre de l'évaluation écotoxicologique de ces nouveaux composés, l'objectif de cette étude expérimentale était d'évaluer l'action de la communauté bactérienne naturelle de l'Estuaire du Saint-Laurent sur le pétrole traité par le nouvel agent anti-adhésif et de comparer avec le pétrole non traité. Un groupe de 4 mésocosmes de 3,5 m^3 chacun a été utilisé pour le suivi à moyen terme de la biodégradation des résidus pétroliers traités et non traités ainsi que pour étudier l'évolution de la flore bactérienne en période estivale. La biodégradation des hydrocarbures dissous dans la colonne d'eau et la microcouche de surface, des émulsions de type eau-dans-huile ainsi que des résidus recueillis dans les trappes à sédiment a été suivie par chromatographie en phase gazeuse, à l'aide de plusieurs rapports d'hydrocarbures, dans les fractions aliphatiques et aromatiques. Dans chaque réservoir, les bactéries hétérotrophes viables (comptages sur plaques et par la technique du Most Probable Number), les bactéries spécifiques du pétrole (MPN) ainsi que les bactéries totales (comptages directs en épifluorescence) ont été régulièrement mesurées. Ces résultats préliminaires permettent de mieux prévoir le devenir et les effets du pétrole traité par ces nouveaux produits suite à un accident pétrolier dans l'estuaire du Saint-Laurent.

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*POTENCIES OF MIXTURES OF POLYCHLORINATED DIBENZO-*DIOXINS (PCDDS) FOR INDUCING ETHOXYRESORUFIN-O-DEETHYLASE (EROD) ACTIVITY IN RAINBOW TROUT*

Single oral doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) or 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) raised EROD activity in a dose dependent manner over 2-16 days. Threshold oral doses for elevating liver EROD activity significantly above control fish were 0.072, 0.25 and 0.74 $\mu g\ kg^{-1}$ for TCDD, HxCDD and HpCDD, respectively. Liver concentrations of the three PCDDs at the threshold of EROD induction were 16, 39 and 350 pg g^{-1}. Slopes of the dose-response curves (EROD activity versus oral dose of PCDD, and EROD activity versus liver concentration of PCDD) were different for the three PCDDs, with slopes decreasing with increasing chlorination. Because of the different slopes, the relative potencies of HxCDD and HpCDD to TCDD decreased with increasing dosages. Problems occur predicting the effects of PCDD mixtures if relative potencies and toxic equivalent factors (TEFs) differ with dose. Using the TEF approach, additivity of mixtures is currently assumed for PCDDs. To test this hypothesis, pairs of PCDDs were tested for additivity. Equipotent mixtures of TCDD with either HxCDD or HpCDD showed less-than-additive or additive actions for inducing EROD activities, as did mixtures where one compound contributed one-third, and the other contributed two-thirds to the EROD activity.

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A TEST OF THE EQUIPOTENCY OF INTERNAL BURDENS OF NINE NARCOTIC CHEMICALS USING DAPHNIA MAGNA

The hypothesis of constant tissue residues of narcotic organic chemicals is tested by determining the effect of body length, time, and ambient concentration on tissue concentration in *Daphnia magna* narcotized by exposure to toxic levels of nine, ^{14}C-labelled, narcotic organic compounds : acetone, acetophenone, benzene, butanol, 1,2-dichlorobenzene, 2-methylnaphthalene, 1,1,2,2-tetrachloroethane, 1,2,4-trichlorobenzene, and toluene. The average tissue concentration of immobilized *Daphnia magna* was 3.1 mmol/kg (95 % CL=1.1 to 5.1), but ranged from 0.15 mmol/kg for 2-methylnaphthalene to 200 mmol/kg for butanol. When all chemicals are pooled in analysis, time (seconds to days) had no effect on the effective tissue concentrations of narcotics, body size (0.4-3mm) had a small but significant effect, and ambient concentrations (1/10th to 48h LC50) had the strongest effect. When the chemicals were analyzed separately the influence of duration of exposure, body size, and ambient concentration varied.

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VARIATION SPATIO-TEMPORELLE DES HYDROCARBURES AROMATIQUES POLYCYCLIQUES DANS LE FLEUVE SAINT-LAURENT

Des échantillons d'eau et de matières en suspension ont été prélevés entre août 1990 et novembre 1991 selon la méthode lagrangienne dans le fleuve Saint-Laurent entre Cornwall et Québec. Les échantillons ont été analysés pour 12 hydrocarbures aromatiques polycycliques.

Les concentrations de HAP totaux dans la phase totale (eau + matières en suspension) varient entre 0,95 ng/L et 55,85 ng/L, avec une teneur moyenne de 15,09 ± 13,20 ng/L. Les concentrations les plus élevées ont été mesurées au printemps et à l'automne. Les fortes concentrations mesurées au printemps peuvent résulter de la libération des HAP accumulés dans la neige pendant l'hiver et de l'augmentation du ruissellement de surface. Les HAP dans les échantillons d'avril 1991, de mai 1991 et de juin 1991 sont presqu'entièrement dans la phase particulaire, suggérant que pendant cette période ils sont introduits dans le milieu aquatique avec les particules. A l'automne, la plus grande fréquence des précipitations entraînerait une augmentation du ruissellement, augmentant ainsi les concentrations de HAP dans le fleuve. Les précipitations peuvent également contenir des HAP avant d'atteindre le sol. Dans l'ensemble, les concentrations augmentent de Cornwall à Québec, allant de 9,10 ± 4,63 à 30,93 ± 17,16 ng/L.

Les charges varient entre 0,30 kg/j et 58,53 kg/j, avec une moyenne de 13,23 ± 14,09 kg/j. La grande variation dans les données est liée à la variation du débit et de la concentration. Les charges les plus élevées sont mesurées en avril 1991 (x = 34,0 ± 15,01 kg/j) et en mai (x = 23,72 ± 14,22 kg/j). La charge moyenne augmente de Cornwall (1,91 ± 1,16 kg/j) à Québec (24,12 ± 20,29 kg/j). Cette augmentation est à la fois due à l'augmentation de la concentration et du débit.

Les trois composés prédominants en terme de concentration sont le phénanthrène, le fluoranthène et le pyrène. Ces composés représentent en moyenne, respectivement, 33,8 %, 24,9 % et 18,2 % de la somme des 12 HAP.

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COMPOSANTES ÉCOLOGIQUES ET TOXICOLOGIQUES DE LA STRUCTURE DU MACROBENTHOS : ÉTUDE DE CAS AU LAC SAINT-FRANÇOIS (QUÉBEC)

L'hétérogénéité spatiale du macrobenthos lacustre dans les écosystèmes anthropisés est reliée aux effets simples et combinés des facteurs écologiques et toxicologiques. Toutefois, la contribution respective de ces différents processus reste à élucider. Dans le cadre d'une étude pilote réalisée en 1989 au lac Saint-François, nous avons déterminé les effets simples et combinés des facteurs écologiques (granulométrie des sédiments, profondeur, trophie, type d'herbiers) et des facteurs toxicologiques (métaux traces, BPCs, HAPs) sur la structure du macrobenthos et de l'indice ICI (Invertebrate Community Index). Le macrobenthos du lac Saint-François se caractérise par deux types de communautés associés au gradient est-ouest et variant par leur densité et leur tolérance vis-à-vis de la pollution organique. L'indice ICI varie de 7 à 27. Les facteurs écologiques et toxiques expliquent moins de 40 % de la variation observée dans la structure du macrobenthos et dans l'indice ICI. L'influence directe des facteurs écologiques est généralement faible (4 à 16 %) tandis que celle des facteurs toxicologiques est plus importante (12 à 29 %). Les effets interactifs des deux types de facteurs expliquent 4 à 14 % des variations observées dans le macrobenthos et l'indice ICI.

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CONCENTRATION EN Hg TOTAL ET MeHg DANS LA MATIÈRE PARTICULAIRE EN SUSPENSION DU RÉSERVOIR DE LG-2

Afin d'évaluer le rôle de l'érosion de la matière organique inondée dans les zones peu profondes des réservoirs quant au relargage du Hg, une baie du réservoir LG-2 a été artificiellement brassée. Suite à la perturbation, des pertes importantes en MeHg dans l'horizon organique des sols inondés ont été enregistrées. Parallèlement, une augmentation importante des concentrations en Hg total et MeHg a été observée dans le seston. Néanmoins ce phénomène est demeuré très localisé et n'a occasionné aucun changement significatif dans les teneurs en Hg des organismes locaux.

Les concentrations en Hg total et MeHg des matières en suspension du réservoir LG-2 s'échelonnent respectivement de 249 à 943 ppb (ng/g sec) et de 8 à 89 ppb. Les concentrations mesurées pour un lac naturel voisin (lac Duncan) sont beaucoup plus faibles, soit de 103 à 316 ppb en Hg total et de 1.3 à 7.2 ppb en MeHg. Ces faibles teneurs reflètent probablement les charges restreintes en carbone et azote du seston du lac naturel, alors que ces éléments sont connus pour leur grande affinité pour le Hg. La proportion de MeHg par rapport au Hg total est également supérieure en réservoir avec une proportion moyenne d'environ 10 % par rapport à seulement 1.4 % pour le lac. Les concentrations en MeHg des particules en suspension sont maximales durant le mois d'août et traduisent l'intense activité bactérienne durant cette période.

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DATA GAPS AND ISSUES ASSOCIATED WITH ASSESSMENT OF PRIORITY METALS IN CANADA

The Canadian Environmental Protection Act (CEPA) Priority Substances Assessment Program is a major federal initiative aimed at assessing the entry, exposure and effects of priority chemicals on ecosystems to determine whether control measures are required to mitigate any adverse impacts. The first Priority Substances List (PSL) included four metals: cadmium, arsenic, chromium, and nickel. Data required to assess priority metals include environmental releases, environmental fate and concentrations, and ecotoxicological data. Over the course of these metal assessments a number of common issues challenging the ecotoxicological research community remained including: 1) availability of reliable concentration data nationally; 2) availability of toxicity data from studies where exposures were quantified; 3) natural vs anthropogenic presence (e.g., backgroud levels); 4) speciation; 5) bioavailability and 6) analytical detection and standardized toxicity testing methods. In this paper, these will be explored using examples from the 4 metals to generate discussion and recommendations to help focus research needs.

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MODELLING POPULATION EXPOSURE-RESPONSE FUNCTIONS FOR USE IN ENVIRONMENTAL RISK ASSESSMENT

Effective environmental management requires accurate prediction of the probable individual, population and ecosystem responses associated with environmental hazards. While much is known about the short-term physiological impacts of toxicants at the individual level, little is known about the long-term responses of populations. This occurs, in part, because of the costs and difficulties associated with completing long-term longitudinal studies. In the absence of such field data it is argued that modelling both bridges the existing information gap and provides a credible means of predicting the long-term population responses. An individuals based Atlantic salmon (*Salmo salar*) population dynamics model, adjusted to include laboratory-derived acutetoxicity data, is used to measure recovery time in a population subjected to concentrations and durations of copper exposure characteristic of an accidental release of mine tailings. Selected recovery criteria are proposed and discussed in terms of their suitability for use in environmental risk assessment. The resulting model data are used to estimate population exposure-response functions and, for purposes of environmental risk assessment, to describe the cumulative frequency distribution of in-situ environmental damage. The output of the model suggests a recovery time of 15 to 20 years and significant increases in the variability of post-perturbation population levels.

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SEDIMENT QUALITY AND RELATED EFFECTS ON BENTHIC ORGANISMS NEAR MINE SITES

It is well established that bottom sediments in water bodies located near mine sites have been historically enriched with various metals due to the settling of particulate matter present in the mine effluent discharges. Effects on the benthic communities are however quite variable depending on numerous factors.

A review of the available information from some Noranda sites on sediment/benthos relationships, along with a limited sampling and testing program near a few Noranda operations, were undertaken to assess the effects of elevated metal levels in sediments, particularly in view of sediment quality guidelines.

Detailed sediment analyses (bulk solid-phase chemistry, porewater analysis, sequentially extracted metal phases, and petrographic analysis) were carried out in parallel with the benthic responses (sediment toxicity testing and benthic community assessment).

The results of this study along with a review of the available sediment quality guidelines suggest that total metal concentrations in the sediments can determine low and severe effects on the benthic community on a site-specific basis, but cannot necessarily be extrapolated to other sites with different characteristics. More work is needed in order to better understand the role of other parameters in regulating toxic effects of metals to the biota.

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AROMATIC DNA-ADDUCTS IN BELUGA WHALES FROM McKENZIE DELTA AND GULF OF ST.LAWRENCE

Polycyclic aromatic hydrocarbons (PAH) are recognised as common environmental pollutants, and many of them, such as benzo(a)pyrene, are potent carcinogens. Environment PAH, including benzo(a)pyrene are metabolized by mammals and fish, and their metabolites can form covalent DNA-carcinogen adducts which can be detected in exposed organisms.

Aromatic DNA-adduct levels in different tissues of beluga whales (*Delphinapterus leucas*) from St.Lawrence estuary and McKenzie delta were determined by ^{32}P-postlabelling technique for retrospective analysis of the effects of PAH in the aquatic environment. The results unequivocally confirm bioactivation and the formation of DNA-adducts and establish the utility of the technique for molecular dosimetry. The presence of the adducts in whales from clean areas suggests the existence of non-pollution related mechanisms for genetic damage in the animals. The post-labelling technique for determination of DNA-adducts and the feasibility of using the DNA-adducts as bioindicators of contaminant exposure will be examined.

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ALTÉRATIONS DES NIVEAUX DE CALCIUM CYTOSOLIQUE LIBRE ET DU pH INTRACELLULAIRE CHEZ LES HÉPATOCYTES DE TRUITE PAR LE TRI-N-BUTYLÉTAIN (TBT) : ANALYSE PAR CYTOMÉTRIE DE FLUX

Le tributylétain (TBT) a été longuement utilisé comme biocide dans les peintures antisalissures pour combattre la prolifération des organismes marins qui s'incrustent sur les coques des navires. Leur toxicité étant maintenant reconnue, la présence de ces composés dans l'environnement constitue une menace sérieuse pour la santé des organismes qui y sont exposés.

Il existe de plus en plus d'évidence démontrant que les perturbations de l'homéostasie ionique joueraient un rôle critique dans la mort cellulaire causée par les substances toxiques. Le but de ce présent travail est d'étudier les effets du TBT sur l'homéostasie ionique, particulièrement au niveau du calcium cytosolique libre (Ca^{2+}_i) et du pH intracellulaire (pH_i) sur les hépatocytes de truite isolés. Le Ca^{2+}_i, le pH_i et la viabilité ont été analysés par cytométrie de flux.

Le traitement des hépatocytes au TBT cause une perte de viabilité qui dépend du temps et de la concentration. Les résultats montrent que le TBT induit une hausse soutenue du Ca^{2+}_i dans ces cellules avant que la perte de viabilité ne soit détectable. De plus, le TBT induit une rapide et importante diminution du pH_i. Ces résultats suggèrent que l'altération de l'homéostasie ionique est impliquée dans le mécanisme de toxicité du TBT chez les hépatocytes de truite.

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L'INTÉGRITÉ BIOTIQUE DES ÉCOSYSTEMES FLUVIAUX

Le ministère de l'Environnement du Québec met en place un réseau de suivi biologique des principales rivières du Québec méridional. Ce réseau, basé entre autres sur les caractéristiques des populations piscicoles, permettra d'évaluer l'état de santé de nos cours d'eaux et de vérifier éventuellement si les efforts déployés en assainissement urbain, industriel et agricole permettent leur régénération biologique.

Dans une première approche, des pêches expérimentales effectuées sur la rivière L'Assomption démontrent pour les secteurs les plus pollués : (1) une baisse importante du nombre d'espèces de poissons; (2) une dominance des espèces reconnues comme tolérantes à la pollution; (3) une absence ou une faible représentativité des espèces intolérantes; (4) un déséquilibre de la chaîne trophique, la densité des espèces omnivores augmentant au détriment des insectivores et des piscivores; (5) une fréquence élevée des poissons affectés par des pathologies externes.

En intégrant l'information précédente, on peut former un indice d'intégrité biotique qui est une expression synthétique de la santé de l'écosystème aquatique. Appliqué à la rivière L'Assomption, l'indice démontre que l'écosystème est en bonne santé dans tout le secteur amont de la ville de Joliette. Par contre, en aval de cette agglomération jusqu'à son embouchure, l'intégrité de l'écosystème est de pauvre à très pauvre pour près de 40 kilomètres. Les rejets non traités de la ville de Joliette sont, pour une large part, responsables de cette dégradation. Les apports de polluants en provenance de tributaires à forte vocation agricole affectent également la santé de la rivière.

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DÉVELOPPEMENTS DE MÉTHODES POUR LES TESTS DE TOXICITÉ SUR LES LIQUIDES ET LES SÉDIMENTS EN UTILISANT DES INVERTÉBRÉS MARINS PROVENANT DES RÉGIONS ARCTIQUES DE L'AMÉRIQUE DU NORD

Des procédures pour des tests de toxicité utilisant certaines espèces d'invertébrés (amphipodes et mysidacés) marins de l'Arctique, sont en cours de préparation. L'objectif est de développer des tests biologiques représentatifs et pratiques pour effectuer le suivi de substances déversées (effluents, lixiviats, sédiments) dans l'environnement marin de l'Arctique. Les espèces pouvant être utilisées lors des tests ont été sélectionnées d'après une revue de la littérature et des discussions avec des experts. Plusieurs fournisseurs du nord ont été identifiés et ont fourni des organismes et des échantillons provenant de l'Arctique pour développer les procédures. Les organismes sont maintenus dans un système en circuit fermé à température contrôlée et les tests sont réalisés dans un incubateur. Les données sur les tolérances environnementales (e.g. température, photopériode, taille des particules des sédiments) et sur les comportements ont été utilisées afin de sélectionner les organismes se prêtant le mieux aux procédures de tests. Des procédures préliminaires et des résultats de tests de toxicité aiguë des sédiments utilisant des amphipodes fouisseurs et des tests de toxicité aiguë en phase liquide utilisant des mysidacés seront présentés.

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LE SUIVI DES ÉCOSYSTEMES AQUATIQUES DANS L'INDUSTRIE PAPETIERE CANADIENNE : GRANDES LIGNES ET ORIENTATIONS FUTURES

Les premières études de suivi des écosystèmes aquatiques près des usines de pâtes et papiers au Canada datent des travaux effectués par T.W. Beak au début des années 1950. Des études typiques incluent des évaluations des communautés biologiques en aval des usines, ainsi que des études sur le goût et l'odeur de l'eau et des poissons. Le but de ces études était de fournir des données qui servaient à prendre des décidions rationnelles pour déterminer les limites des rejets d'effluents afin de préserver les rivières et les lacs. L'émergence de problèmes nouveaux durant la dernière décennie nous a conduit à nous interroger sur les effets des organo-chlorés sur les écosystèmes aquatiques. Une approche globale du suivi de l'écosystème est maintenant couramment utilisés afin de déterminer les impacts sur les différents niveaux de l'écosystème et d'identifier les relations de cause à effet. L'utilité et les limites d'outils permettant un suivi plus poussé sont discutées, tels les tests de toxicité sublétale, les modèles de prévision de la qualité de l'eau, le processus de la sédimentation, et la bioaccumulation de composés organiques dans la chaîne alimentaire. La nouvelle réglementation canadienne sur les Études de Suivi des Effets sur l'Environnement (ESEE) est brièvement exposée et quelques avantages et inconvénients soulignés. Une revue de certaines études est aussi présentée ainsi que les leçons à en tirer et les orientations qu'elles suggèrent pour l'avenir. Les utilisateurs et les acheteurs de produits de pâtes et papiers exigent aujourd'hui qu'une certaine protection de l'environnement soit appliquée. La meilleure stratégie économique pour l'industrie papetière est d'oublier le passé, de régler les problèmes actuels de manière scientifique et de communiquer ouvertement les découvertes réalisées.

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UN SYSTEME D'ÉCOULEMENT CONTINU A CIRCUIT FERMÉ POUR LE MAINTIEN ET LA CULTURE D'ORGANISMES MARINS PROVENANT DE RÉGIONS TEMPÉRÉES ET ARCTIQUES

Cette présentation décrit la conception, le coût pour la construction, l'opération et l'entretien d'un système en circuit fermé (i.e. eau recirculée) d'écoulement continu d'eau de mer reconstituée pour le maintien et la culture à long terme d'organismes marins à des températures variant de 0° et 15°C. Le système est relativement peu coûteux et requiert peu d'entretien. Il est idéal pour les laboratoires d'écotoxicologie se spécialisant dans l'élevage d'organismes benthiques, épibenthiques et pélagiques utilisés lors d'essais de toxicité en phase liquide ou solide (ex. : sédiments marins). Le système est conçu d'après une revue de la littérature sur les systèmes commerciaux pour la culture d'animaux marins. Il est équipé de filtres mécaniques et biologiques, d'une colonne à fractionnement de mousse, d'une unité de contrôle de la température et d'un dispositif indépendant pour le contrôle de la photopériode. L'entretien du système est simple et nécessite un suivi régulier afin d'assurer une qualité constante de l'eau et la performance de l'équipement.

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BOILER BLOWDOWN TOXICITY REDUCTION TESTS

Boiler blowdown effluent from both nuclear and thermal stations was often toxic to both rainbow trout and *Daphnia magna* in toxicity tests conducted during the monitoring phase of the Ontario Municipal Industrial Strategy for Abatement (MISA) Program. The toxicity of boiler blowdown effluent may have been caused either by its low ionic content or through interactions of the low ionic content and elevated metal concentrations. Accordingly, follow-up studies were conducted to evaluate mechanisms of reducing the toxicity of boiler blowdown effluent. Addition of calcium ions, either by adding calcium chloride ($CaCl_2$) or recirculation over a limestone filter for ≈ 12 h, largely eliminated the toxicity of boiler blowdown effluent to trout, but the treated effluent was still toxic to *Daphnia magna*. Addition of humic acid (≈ 20 mg/L as dissolved organic carbon) effectively mitigated the toxicity of boiler blowdown effluent to both *D. magna* and rainbow trout. The toxicity of boiler blowdown effluent thus appears to result from the interactions of its low ionic content and elevated metal concentrations, but addition of humic acid or humic acid and $CaCl_2$ would effectively reduce this toxicity.

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MERCURY METHYLATION ACTIVITY OF SEDIMENTS FROM MISSISSAGI RIVER RESERVOIRS

Mercury methylation activity, as determined by the production of radiolabeled methylmercury from added $^{203}HgCl_2$, was measured for sediments collected from three Ontario Hydro reservoirs on the Mississagi River. The reservoirs, Rocky Island, Aubrey and Tunnel Lakes, were flooded from 25 - 40 years earlier. Methylation activity (dpm per g sediment) did not differ significantly among reservoirs, but was significantly affected by depth of collection (littoral (< 5 m) versus profundal (> 15 m)). Methylation activity was positively correlated with organic content ($r^2 = 0.44$), however, and the effect of depth was not significant when methylation activity was expressed per g of organic sediment. As methylation activity was positively correlated with organic content of the sediment, measures taken to reduce the organic content of sediments in new reservoirs should also reduce mercury methylation and subsequent accumulation of methylmercury within the aquatic ecosystem. Although addition of a mixed culture of sulphate reducing bacteria (*Desulfovibrio desulfuricans* ATCC 29577, *D. gisas* TVA, and *D. vulgaris* ATCC 29579) and *Halfnia alvei* (TVA) approximately doubled the average mercury methylation activity of sediment samples, the response was sufficiently variable that the differences were not statistically significant.

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REDOX-PROCESSES IN FRESH WATER SEDIMENTS

Sediments, as a rule, are sources of reducing agents in the aquatic system. They influence the dynamics of aquatic redox processes. The investigation of redox properties and nature of the reducing agents is a very important problem.

In this study, we report some chemical properties, redox-processes and methods for testing fresh water sediments.

Reducing activity of sediments are caused mainly by the solid phase, at the same time porewaters exhibit very little reducing activity. We found that the main reducing agent in the solid phase of bottom sediments is biotic generated FeS. As established, both artificial and sedimental amorphous FeS exhibit very high reducing activity with respect to such oxidizing agents as hydrogen peroxide, oxygen, organic dyes, nitro- and nitrozocompounds and so on. Thiols, thiosulfate and other polythionates to be likely can be responsible for little reducing activity of porewater. We suggest that these strong nucleophilic and reducing compounds take main part in anaerobic degradation of organic chloro-, azo-, nitro-, nitrozocompounds and some other pollutants.

Also we developed some methods for measuring amount and activity of the reducing agents in the sediments using potassium ferricyanide, hydrogen peroxide and heteropolyacids.

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ESQUISSE D'UN RÉSEAU DE SURVEILLANCE ÉCOTOXICOLOGIQUE DE LA FAUNE DU SAINT-LAURENT

Dans la foulée des récentes interventions gouvernementales visant la dépollution du bassin du Saint-Laurent, nous avons entrepris la collecte de spécimens de la faune vivant à proximité des industries les plus polluantes installées le long de cet important cours d'eau. Ces organismes, pour la plupart des oiseaux (piscivores, molluscovores et insectivores) auxquels s'ajoutent une espèce d'amphibien et de reptile, font présentement l'objet d'une évaluation de leur potentiel de bioindication. Une fois les analyses écotoxicologiques complétées, nous saurons quelles espèces de vertébrés supérieurs devraient faire partie d'un réseau de surveillance intégré de contamination des écosystèmes du Saint-Laurent.

Une bonne partie de l'information écotoxicologique provient des oeufs. Des jeunes, davantage représentatifs de la contamination locale sont aussi récoltés. Nous profitons de cette étude pour mettre au point des techniques de dépistage (résidus de contaminants et biomarqueurs physiologiques) qui permettront de connaître la contamination et la condition des oiseaux à l'aide des téguments et du sang, deux tissus qui n'exigent pas de sacrifier d'animaux.

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RODRIGUE[1] J., DESGRANGES[1] J.L., TITMAN[2] R.

USE OF PEKING DUCKS (ANAS PLATYRHYNCHOS) AS A BIOINDICATOR OF CONTAMINATION BY BIOACCUMULABLE SUBSTANCES IN THE NATURAL ENVIRONMENT (ST.LAWRENCE RIVER, CANADA)

Eighteen-month-old adult female Peking Ducks (*Anas platyrhynchos*) were placed in nine locations (covering 375 km of river) in the freshwater portion of the St.Lawrence River for periods varying from 14 to 73 days during the summers of 1987, 1988 and 1989. Two stations located in a fluvio-lentic portion of the river (Lake St. François) with different PCB concentrations in their sediments were selected for establishing a time-curve for the accumulation of contaminants that bioaccumulate in the liver. Four individuals were taken from each site at regular intervals (average of 15 days) throughout the duration of the study.

The ducks rapidly lost weight during their first 20 days in the natural environment but stabilised after about 40 days, or some 20 days after they had started feeding from the local food supply.

There was a rapid increase in the number and concentration of contaminants detected in the livers of ducks exposed to pollutants in the natural environment. These individuals were generally from 10 to 1,000 times more contaminated than the control ducks. The number and concentration of contaminants detected depended on: 1) contamination of the site, 2) exposure time of the ducks and 3) capacity of the ducks for metabolizing these compounds. No significant difference was found between the time-curves for the accumulation of contaminants at the two stations in Lake St.François.

The use of domestic ducks as bioanalytical probes has several advantages. They can be obtained readily and cheaply, and the sex and age of the individuals can be controlled. Since they are white and not very mobile, thus easy to locate, they provide quality information on the contamination of specific sites. They also permit monitoring of the ducks' bioaccumulation of a number of xenobiotics, including heavy metals and organochlorine compounds. The use of Peking Ducks does, however, have some limitations and disadvantages, such as the need for appropriate habitats to ensure their survival, and the considerable stress caused by transferring the ducks from the breeding farm to the natural environment, thereby obliging them to search for food and exposing them to predators and poachers, two totally new experiences for them.

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LA MACROAUTORADIOGRAPHIE : UN OUTIL POUR LES ÉTUDES DE TOXICOLOGIE ENVIRONNEMENTALE

Un des problèmes auquel les chercheurs sont confrontés en voulant évaluer la toxicité d'un composé chimique et élucider les mécanismes de cette toxicité est la possibilité que le site d'accumulation dans les organismes vivants soit tout à fait inattendu ou localisé dans une structure anatomique trop petite, fragile, diffuse ou difficilement accessible. Il est toujours possible d'examiner un à un tous les tissus d'un organisme vivant, mais cela n'est pas pratiquement possible.

La macroautoradiographie donne la possibilité de contourner cette difficulté en permettant de visualiser rapidement et en détail la distribution d'un composé chimique marqué avec un isotope radioactif dans tous les tissus d'un organisme vivant. Cette technique consiste à faire des cryosections fines (20-60 µm) d'un animal entier (poisson, souris, étoile de mer) auquel a été administré (injection intraveineuse ou péritonéale, contact direct, nourriture) un composé radioactif. Ces sections sont ensuite mises en contact avec un film radiographique pour une période de quelques semaines à quelques mois. L'image ainsi obtenue permet de localiser précisément le composé chimique administré à l'animal. La résolution de l'image est telle que des détails de moins de 100 µm peuvent être visibles.

Mise au point en Suède au début des années 50, la macroautoradiographie a surtout été utilisée en recherche médicale et pharmaceutique. L'application de cette technique lors de nos travaux sur l'accumulation d'ions métalliques et de composés organométalliques chez la truite et l'étoile de mer a démontré que la macroautoradiographie peut aussi être très utile en toxicologie environnementale, bien qu'elle soit très peu utilisée à cette fin. La macroautoradiographie facilite l'orientation des recherches en mettant rapidement les chercheurs sur la bonne voie. C'est une technique simple et rapide qui peut donner de nombreuses informations très difficiles à obtenir autrement.

C'est une technique de choix à considérer pour l'évaluation environnementale des nouveaux composés chimiques avant leur lancement sur le marché... ou dans l'environnement.

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ALUMINUM COMPLEXATION BY A NATURALLY OCCURRING FULVIC ACID : DETERMINATION OF CONDITIONAL STABILITY CONSTANTS AND PREDICTION OF INORGANIC AL LEVELS IN SYNTHETIC ACIDIC SOFT WATERS

During periods of snowmelt, rivers on the Canadian Shield are subjected to a pulse of acidic water, including increased levels of Al and dissolved organic carbon (DOC). Dissolved Al levels in Quebec's North Shore rivers, important habitats for Atlantic salmon (*Salmo salar*), can attain concentrations of 11 μM during early spring (Campbell et al., 1993 : CJFAS 49 : 1938-52). A significant proportion of this Al is complexed with DOC and these complexes do not appear to be implicated in the toxicity of Al observed with fish. In the context of toxicity bioassays, batch titration studies were conducted to determine the complexation behaviour of Al in synthetic soft waters, representative of those present during snowmelt on the Canadian Shield.

Experiments were conducted with a natural soil-derived fulvic acid (FA) and a reconstituted soft water, at 10° C. Studies involved three fulvic acid concentrations at pH 5.0 ([FA] = 5,10 and 20 mg L^{-1}) and a single FA concentration at pH 4.5 (10 mg L^{-1}); Al concentrations ranged from 0 to 16.7 μM. Measurements of Al speciation involved an automated colourimetric method to differentiate among total (acid reactive) Al, organic monomeric Al and inorganic monomeric Al. FA concentrations were verified by absorbance measurements at 400 nm. Conditional stability constants (K_{cond}) were determined from Langmuir isotherm plots of the data; these constants were used with the computer program MINEQL$^+$ to calculate the speciation of Al. The effects of varying pH and FA levels on the concentration of inorganic Al will be discussed in the context of designing appropriate bioassay media.

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AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF CHLORINATED WASTEWATER EFFLUENT DISCHARGES FROM FOUR SEWAGE TREATMENT FACILITIES IN THE ATLANTIC REGION

The final effluent from four sewage treatment facilities was chemically characterized and evaluated for acute and sub-lethal aquatic toxicity. The impact of chlorinated effluent discharges on the benthic macroinvertebrate community was determined at two treatment facilities discharging to freshwater environments and one facility discharging to a marine environment. GC-MS screening identified a wide variety of non-chlorinated compounds and some phenolic chlorinated compounds in the effluents. The acute toxicity of the effluent to rainbow trout (*Oncorhynchus mykiss*), threespine stickleback (*Gasterosteus aculeatus*), *Daphnia magna* and *Photobacterium phosphoreum* was more pronounced with effluent from plants which had mean total residual chlorine concentrations above 0.2 mg/L. Abnormal shell development in larval oysters (*Crassostrea gigas*) was observed at concentrations as low as 0.2 % after a 48-h exposure to effluent from one plant. All effluents tested were not toxic to the alga *Selenastrum capricornutum* and the cladoceran *Ceriodaphnia dubia* and were not mutagenic. A statistically significant decrease in diversity of benthic macroinvertebrates was observed at sampling stations less than one metre from the effluent outfall of all plants and at 50 metres downstream of the outfall of one plant discharging to a brook. It was not possible to determine whether residual chlorine or other substances contributed to the observed effects on benthic macroinvertebrate diversity at the three sites.

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ST-LOUIS R.[1], PELLETIER[1] E., MARSOT[1] P., FOURNIER[1] R.

SOME ASPECTS ON THE ROLE OF A MARINE MICROALGA IN THE FATE OF TRIBUTYLTIN IN MARINE WATERS

It is well established that microalgae can accumulate toxic compounds such as heavy metals, PCBs and organometallics. This fact has two ecological consequences : 1) accumulated toxic chemicals can be biotransferred through the trophic web; 2) the biodegradation of organic and organometallic xenobiotics can occur by biochemical processes inside the cells and algae could become a sink for these compounds. The latter consequence takes place only if the physiological mechanisms involved are not inhibited and the extent of one consequence over the other one depends on the coupling of absorption vs adsorption processes. We investigated the mechanism of accumulation of a toxic organometal, tributylin (TBT), by the marine microalga *Pavlova lutheri*. Exposures to TBT, at different concentrations, were realised on algae grown by two techniques : continuous flow and batch cultures. Results revealed : i) *P. lutheri* is much more tolerant to TBT in a continuous flow system; its growth rate is not affected by a TBT concentration of ca. 5 $\mu g.l^{-1}$ whereas it decreased significantly in a batch culture system when exposed to ca. 0.1 $\mu g.l^{-1}$; ii) degradation of TBT is more important in the continuous flow system compared to the batch culture for the same period of exposure; iii) the intracellular accumulation of TBT increases with contamination of culture medium to a maximum level; thereafter, the absorption of TBT decreases to a plateau even when aqueous TBT concentrations still increase. The adsorption of TBT on cell external walls follows a linear relationship with water contamination. These trends are observed for both culture systems. The environmental implications of these observations on the mechanism of algae TBT accumulation and biotransfer are discussed.

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WETLANDS AS IMPORTANT SOURCES OF METHYL MERCURY TO BOREAL FOREST ECOSYSTEMS

We measured precipitation inputs and export of methyl mercury from several types of relatively pristine boreal catchments in NW Ontario dominated by uplands, lakes, or wetlands. Wetlands were important sources of methyl mercury to boreal forest ecosystems. Yields of methyl mercury per unit area were 79 times higher from wetlands (5.6 mg.ha^{-1}. yr^{-1}) than from uplands (0.07 mg.ha^{-1}. yr^{-1}). Upland catchments were sinks for atmospheric input of methyl mercury which was estimated to be 0.15 mg. ha^{-1}.yr^{-1}.

We experimentally flooded a small wetland at the Experimental Lakes Area in NW Ontario by raising the water level 1.2 m to study effects of flooding due to reservoir construction. Water concentrations of methyl mercury increased dramatically in the new reservoir immediately following flooding of the wetland. Methyl mercury concentrations in the new reservoir are continuing to be measured and will be presented at the workshop.

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INHIBITION OF CADMIUM AVOIDANCE WITH LIGHT : A COMPETING GRADIENTS PARADIGM

Most preference-avoidance testing of contaminants is conducted under simplified, uniform environmental conditions with the contaminant as the only directive factor (*sensu* Fry 1947). However gradients of temperature, light, oxygen, etc. are usually present in natural settings, and can overlay those of pollutants. Fish often show strong preferences for narrow ranges within such natural gradients. When the directing influence of contaminant and natural environmental gradients affect a fish simultaneously, innate responses to natural factors may inhibit avoidance of the anthropogenic chemical. Thus conventional avoidance testing may overestimate the influence of pollutants in directing fish movement. While a few studies have tested responses to pollutants using a competing gradients paradigm, none have examined how light preferences can alter avoidance responses to metals. Light is an important cue for fish in habitat selection. Most species respond to light gradients in a photopositive or photonegative manner at some point during their life history. We tested whether a simple light gradient could alter the avoidance responses of lake whitefish (*Coregonus clupeaformis*) to cadmium. When presented with a choice between high (1400 lux) and low (6 lux) light levels in a counter-current test tank, whitefish strongly preferred the low-light region. When cadmium was added to this preferred side at increasing increments, fish did not abandon it until a concentration 50 times that otherwise avoided was reached.

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GROUNDWATER ISSUES IN THE ASSESSMENT OF PRIORITY SUBSTANCES IN CANADA

The assessment of a priority substance under CEPA requires a holistic ecosystem approach, in which the entry, exposure, and effects of the substance in each environmental medium are considered. Groundwater is not only an ecosystem in itself, but also an integral part of the hydrologic cycle. Several PSL 1 assessments included substances where groundwater contamination was an important component. An assessment of groundwater contamination should, therefore, cover the potential for effects on the groundwater ecosystem, and the potential for effects on aquatic and terrestrial biota via contaminated groundwater discharge. The evaluation and use of Canadian groundwater data in assessments of priority substances posed several challenges because of a lack of information and understanding on: 1) groundwater mapping and characterization in Canada (e.g., aquifer size, location, movement etc...); 2) nature and extent of groundwater contamination; 3) groundwater ecosystems and effects that contaminants may have on them; 4) hydrologic relationship between groundwaters and surface waters in Canada (e.g., pollutant transport and surface water recharge); and 5) impacts of contaminated groundwater on surface water and terrestrial ecosystems. This presentation will identify groundwater research needs encountered during the national assessments of several volatile organic chemicals.

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TOXICITY CAUSED BY DISBALANCE OF REDOX PROCESSES IN NATURAL WATERS

Hydrogen peroxide is a carrier of active oxidizing equivalents. This stipulates the necessity of its presence in natural water systems to ensure their biological full value. Pollution of aquatic environments can be accompanied by disappearance of hydrogen peroxide, disbalance of redox-processes and ead to formation of quasi-reductive and super-oxidative conditions, which can be toxic for aquatic biota. The death of adult fishes occurs under super-oxidative conditions, while the death of larvae - under the quasi-reductive ones. The formation of super-oxidative conditions results from the excessive intensification of free-radical processes (as a result of photoinitiation, radioactive contamination, etc.), especially, in the presence of higher concentrations of manganese and chromium ions. Metal ions in the super-oxidative state (and another active particles) are accumulated on gills and destroy it. In this work we consider the change of aquatic redox-state caused by high production by certain species of microalgae of reducing agents which effectively interact with hydrogen peroxide and are toxic for fish larvae. We consider too the role of sunlight and microalgae in regulation of hydrogen peroxide content in natural waters. Catalytic redox-detoxication of natural waters has been suggested for fish breeding.

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METABOLISM OF POLYNUCLEAR AROMATIC HYDROCARBONS BY BROWN BULLHEAD

We have investigated the metabolism of three polynuclear aromatic hydrocarbons (PAHs), namely benzo(a)pyrene (BaP), chrysene and phenanthrene by liver microsomes of brown bullhead (*Ictalurus nebulosus*), a bottom-dwelling fish known to be susceptible to the carcinogenic action of PAHs. We have also examined the formation and persistence of DNA adducts in the liver of brown bullhead treated with BaP.

Among the three PAHs, BaP was metabolized at the highest rate (38.1 pmol/mg protein/min), chrysene at an intermediate rate (30.2 pmol/mg protein/min) and phenanthrene at the lowest rate (14.1 pmol/mg protein/min) by liver microsomes from untreated bullheads. Our data show that phenanthrene is a rather poor substrate for bullhead liver microsomes in contrast to rat liver microsomes for which phenanthrene has been reported to be the best substrate among the PAHs studied.

Dihydrodiols and phenols were the major classes of metabolites formed from the three PAHs by the liver microsomes. The liver microsomes metabolized the three PAHs more efficiently at the benzo-ring than at the K-region, thus showing comparable regiospecificity. Compared to bay-region diols, diols with a bay-region double bond (putative proximate carcinogens) are formed in a greater proportion from all the three PAHs. The major DNA adduct in the liver of brown bullhead treated with BaP was anti-BaP-7,8-diol-9,10-epoxide-deoxyguanosine, which persisted for several weeks after treatment. Supported by U.S. EPA Grants R-815621 and R-813799.

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THE IML BENTHOCOSM : A MESOCOSM FACILITY FOR THE STUDY OF DEEP SOFT BOTTOM BENTHIC ENVIRONMENTS

Bulk concentration measurements are probably inadequate for the evaluation of toxicity of a chemical substance in dynamic living marine sediments. There may be chemical alterations over small depth intervals due to diagenetic chemical zonation and benthic fauna are significant transporters of dissolved and particulate substances both vertically and laterally within the sediments, and create considerable heterogeneity. Most benthic ecology information has been garnered from intertidal or shallow marine environments and biogeochemistry studies are rare for any sedimentary milieu. For the deeper environments susceptible to toxic substance contamination, modern mud accumulating basins, such as the 350 m-deep Laurentian Trough in the Gulf and Lower Estuary of the St.Lawrence, the logistics of monitoring the natural sediment are daunting. Having a portion (a mesocosm) of the seafloor in the laboratory could provide continuous access for observation and controlled experimentation with toxic substances that we cannot simply add to the real environment.

Encouraged by the success of researchers at the Solbergstrand facility in keeping mud-dwelling fauna from the Oslofjord alive even after depressurisation of about 25 atmospheres, and the relatively inexpensive "boxcosm" studies at NIOZ, a pilot project, now in its third year, was begun at IML to see if the Laurentian Trough benthos was also amenable to laboratory preservation and study. Four experiments have shown that this is quite feasible, as indicated by continuous mega-and macrofaunal displacement, feeding and burrowing, with at least 50 % survival; and with aerobic bacterial numbers and activity and whole sediment oxygen demand similar to fresh sediment.

The benthocosm facility consists of three 1m X 1m, insulated, double-walled, Gel-coated fiberglass basins with covers. A single half H.P. titanium chiller-pump is adequate to circulate 4.5° C water through the double walls and bases of all three basins. Additional ¼ H.P.

units are used to slowly circulate previously collected bottom water, input through submerged diffusers and drained at the water surface, between each basin and a continuously cooled reservoir. Particle filters and UV irradiation lamps can be brought inline as desired. The closed-system design is unfortunate but necessary, since bottom water is too distant, collection is laborious and cannot be done during the winter. Experimentation with salt addition to readily available filtered seawater from 17m depth offshore, has revealed no obvious reaction or deterioration in the benthocosms and continuous or periodic replacement with such water may prove useful in preventing buildup of undesired dissolved components. Another necessary compromise is that the overlying water oxygen concentrations quickly approach saturation in the benthocosms, a marked difference from the natural condition of less than 25 % saturation. This results in a doubling of the oxygen penetration depth to about 20 mm.

Direct observation has already shown that the brittle star *Ophiura sarsi* and the mud star *Ctenodiscus crispatus* are extremely effective in redistributing all of the material in the upper few mm of the sediment, and casts of the galleries of the burrowing shrimp *Calocaris templemani* reveal liter volumes/m^2 of subsurface water which must be frequently exchanged to remain oxygenated. Time-lapse video is also being used to study periodicities of surface exposure and rates of particle displacement by individual organisms.

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SINOTTE[1] M.

LES OBJECTIFS ENVIRONNEMENTAUX DE REJET POUR LES SOURCES PONCTUELLES ET LE CADRE LÉGAL AU QUÉBEC

Le ministère de l'Environnement du Québec (MENVIQ) s'est fixé deux grands objectifs : préserver la diversité, la productivité et la pérennité des écosystèmes, et diminuer l'exposition des personnes et des espèces aux substances toxiques. Afin d'atteindre ces objectifs, le MENVIQ a développé une approche de protection du milieu aquatique basée sur la protection des usages de l'eau. Cette approche, qui se veut préventive d'un point de vue environnemental, a l'avantage d'être quantitative et de guider les choix d'interventions d'assainissement pour le contrôle des rejets liquides toxiques.

Dans ce contexte, l'exposé a pour but de présenter les grandes étapes de la procédure de définition des objectifs environnementaux de rejet. Voulant protéger ou récupérer les usages de l'eau, la procédure s'appuie sur la protection du milieu aquatique contre les effets toxiques des substances chimiques en exigeant le respect des critères de qualité de l'eau à la limite d'une zone de mélange dans le cours d'eau. Par la suite, le cadre légal de l'application de cette approche sera présenté en insistant sur son utilisation dans le cas de nouveaux établissements industriels et dans le nouveau cadre d'application qu'est l'attestation d'assainissement.

Enfin on procédera à une brève comparaison entre l'approche du MENVIQ et celle proposée par le Great Lakes Water Quality Initiative (GLWQI) pour unifier les méthodes en vigueur parmi les états américains du pourtour des Grands Lacs.

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ÉVALUATION DE LA TOXICITÉ DE COMPOSÉS PÉTROLIERS ET D'AGENTS DE TRAITEMENT AVEC LE TEST SUR PHOTOBACTERIUM PHOSPHOREUM

The Microtox® test, based on the bioluminescence inhibition of the marine bacterium *Photobacterium phosphoreum*, was used to evaluate the toxicity of various oil fractions in seawater. First, toxicities of the water soluble fraction (WSF) of a crude oil and its constituents such as aromatic and polyaromatic hydrocarbons were tested. Toxicity of the WSF was monitored while the evaporation and the photooxidation of oil components were progressing. The Microtox® test seemed to be very sensitive to oil compounds. Some chemicals used for the treatment of oil slicks at sea (dispersants and anti-adhesive agents) were also tested. On the other hand, an experiment conducted in mesocosms (3.5 m^3 of seawater each) allowed us to study the toxicity of various oil fractions that could be found in the marine environment after an oil spill : emulsified/degraded oil residues, surface film, dissolved components in the water column and settling oil fraction. These preliminary results show that the Microtox® test used in combination with mesocosms is an efficient experimental tool to assess under natural conditions the toxicity of crude oil spilled at sea.

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OIL BIODEGRADATION IN COLD SEAWATER - A MESOCOSM STUDY -

A group of five mesocosms (3.5 m^3 each) located at Pointe-au-Père (St.Lawrence Estuary), was used to study the behaviour of crude oil dispersed in cold and icy seawater (-1.8 to 5.5°C) under various environmental conditions. Experiments took place during winter, spring and autumn and lasted from 2 weeks to 2 months. The bacterial response to the oil was assessed by recording the growth of total bacteria, viable heterotrophic bacteria and oil-degrading bacteria. The oil biodegradation was monitored by means of gas chromatography; some hydrocarbon ratios in both aliphatic and aromatic fractions of the oil served as biodegradation indexes. A "Combined Index of Biodegradation" (CIB) defined from the most significant hydrocarbon ratios was proposed to evaluate the overall biodegradation process. The use of mesocosms allowed us to study the biodegradation in various parts of the aquatic environment, at the air-water interface (oil film and emulsions), the water column (dissolved components and dispersed oil droplets) and the settling oil residues. Interactions between bacterial and microalgal communities were also observed in mesocosms. The relevance of these experimental results to an oil spill in the St.Lawrence Estuary is discussed.

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FISH AS INDICATORS OF HEAVY METAL POLLUTION IN THE ST. LAWRENCE RIVER

Bioaccumulation in adult fish has commonly been used in the assessment of toxic chemicals in the St.Lawrence River, but was found to be a poor indicator of heavy metals other than Hg. The use of young-of-the-year fish (Spottail Shiner, *Notropis hudsonius*, and yellow perch, *Perca flavescens*) as an alternative to adult fish was therefore evaluated. The results showed that they can be used successfully as indicators of Hg, Cd, Pb, Cr, and Ni, but not for Cu and Zn. The role of fish as an indicator of heavy metal pollution was further investigated by studying hepatic metallothionein (MT) levels in White Sucker (*Catostomus commersoni*). Feral fish were put into cages placed upstream and downstream of Beauharnois near Montreal, known to be a source of heavy metals. After exposure of about a week, the livers of the fish were dissected out for MT determination. Three experiments were carried out: July, August, and October, 1991. Hepatic metal content was determined on some specimens from the August experiment. Results indicated that there was a direct relationship between hepatic copper and zinc concentrations and MT content, all being higher in specimens from the upstream site. Recent data on sediments from the same site suggest that MT induction in white sucker may be more related to sediment contamination (Cu and Zn) than to the presence of point sources. In conclusion, an evaluation of MT induction in young-of-the-year fish as a possible environmental indicator is strongly recommended.

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A FRAMEWORK FOR THE ASSESSMENT OF SEDIMENT QUALITY IN CANADIAN FRESHWATER ECOSYSTEMS

Information on the chemical composition of sediments is essential for evaluating the relative degree of contamination among sampling areas; however, sediment chemistry data alone do not provide an adequate basis for identifying or managing contaminated sediments. Biological effects-based sediment quality guidelines, derived using data on sediment toxicity and geochemistry, are also required to interpret the significance of these data. Under the auspices of the Canadian Council of Ministers of the Environment, Environment Canada is developing Canadian sediment quality guidelines which can be used across the country to evaluate the relative degree of sediment contamination, with the goal of protecting and maintaining healthy aquatic ecosystems. These guidelines denote concentrations of individual chemicals below which adverse biological effects are not expected. Sediment quality guidelines for freshwater ecosystems support federal, provincial/territorial, and non-governmental programs which encompass environmental assessment activities and are important components of regional Action Plans and the National Contaminated Sites Remediation Program. Biological assessments of sediment quality, including benthic invertebrate community analyses and direct toxicity testing can be used, in conjunction with sediment quality guidelines, to confirm predictions of the likelihood of biological effects. Various methods are also available for assessing the natural background concentration, and thereby determining the degree of anthropogenic enrichment, of sediment-associated chemicals. This paper provides background on Environment Canada's freshwater sediment quality guideline development program and illustrates how Canadian sediment quality guidelines can be used, in conjunction with other interpretative tools, as a basis for effective evaluation and management of sediment quality in freshwater ecosystems.

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BIOLOGICAL TRANSFORMATION OF SEDIMENT PCBS IN THE ST. LAWRENCE NEAR THE MASSENA, NY, SUPERFUND SITE

Chromatographic analyses of thirty one sediment cores taken near three industrial discharge points in the St.Lawrence River (Reynolds Metal, General Motors, and ALCOA) revealed that sediments were mainly contaminated with Aroclor 1248 at concentrations ranging from 1 to 7 000 ppm. Congener profiles in the sediments showed evidence of reductive dechlorination at all sites except Reynolds 001. The extent of dechlorination, as determined by the average number of Cl per biphenyl, varied widely between cores. No correlation was found between sediment PCB concentrations and the extent of dechlorination. Dechlorination occurred primarily from the *meta* position,, but at some sites *para* dechlorination was predominant. No *ortho* dechlorination was found. Therefore, although PCBs are dechlorinated in most anaerobic sediments, their biotransformation appears to be highly heterogenous even within a small area. Laboratory incubation studies, carried out with sediments artificially contaminated with 2,3,- and 2,4,5-chlorobiphenyls (CBPs) and Aroclor 1248 using sediment slurries made from selected cores as inocula, showed that all sites including Reynolds 001 harbored dechlorinating microorganisms. The results also disclosed that the pathway, products, and rate of dechlorination were different from site to site. Therefore, the dechlorinating populations and their activities appear to be site-dependent.

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DESIGN, DEVELOPMENT AND CONSTRUCTION OF A FIELD MICROCOSM RESEARCH FACILITY

This site is located on the property of the Guelph Turfgrass Institute at the University of Guelph close to the University and to appropriate research laboratory facilities. A water supply pond (62 m X 62 m) 4 m deep has been constructed, has been filled with water and is currently undergoing naturalization. A total of 30 pools have been installed. The pools are made from steel supported on a ring of concrete. The base of the pools in filled to a depth of 5 cm with fine sand. They are connected through a 3-way valve to two drain lines, one returning to the pond, the other to a waste line. The liners are made from food grade PVC and are non-toxic to aquatic life. The connections and drain lines are all made from PVC or ABS pipe. The pools were build above ground but have been back-filled to the height of the wall to protect them from low temperatures in the winter. Water is supplied from an irrigation pump via a 10 cm line to locations close to the pools. This allows easy refilling and circulation of water in the pools.

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ECOLOGICAL RISK ASSESSMENT PROSPECTS FOR THE END OF THE 1990s

Ecological risk assessment as a distinct activity is only eleven years old, but it draws upon and combines a 300 year tradition of risk assessment with a 40 year tradition of ecological toxicology. Ecological risk assessment has gained ascendancy over prior less formal and quantitative ecological assessment traditions by riding the coat-tails of human health risk assessment. It will continue to gain influence only by delivering on the analogy to health risk assessment. That is, it must deliver clear and credible characterizations of risks to ecosystems and populations that include both best estimates of specific effects and explicit accounting of uncertainty. The greatest strategic problem facing ecological risk assessors in this decade is how to develop consensus concerning methods and models without inducing the rigidity that often characterizes regulatory health risk assessments. Without a set of default tools, ecological risk assessment will remain a craft with too many masterless apprentices and dilettante customers. However, the diversity of ecosystem structures and functions requires flexibility and attention to the particular site and receptor. Ecological epidemiology and adaptive management provide strategies for dealing with this quandary.

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BIODÉGRADATION DES BPC PAR LES BACTÉRIES PÉRIPHYTIQUES DU FLEUVE SAINT-LAURENT

Des microorganismes capables de transformer les BPC ont été isolés de divers environnements (sol, eaux, boues activées). La voie catabolique des BPC chez les bactéries aérobies est celle qui sert à dégrader le biphényle. Il semble que l'évolution ait doté cette voie catabolique d'une certaine plasticité lui permettant de dégrader plusieurs analogues chlorés du biphényle. Toutefois, seuls les congénères BPC à faible teneur en chlore sont transformés. De plus, à cause du fait que d'un point de vue évolutif les interactions entre la voie catabolique du biphényle et les voies du chlorobenzoate ne sont pas encore suffisamment bien harmonisées, la dégradation non contrôlée importante de métabolites indésirables dont la toxicité reste à évaluer. Sachant que les organismes périphytiques peuvent mobiliser les BPC des sédiments aquatiques, il était intéressant de vérifier la présence dans cette population de bactéries potentiellement capables de dégrader les BPC. Nous avons développé une méthode de culture du périphyton sur membrane de téflon et nous l'avons appliquée à l'étude de la microflore périphytique du fleuve Saint-Laurent. Bien que nos travaux aient mis en évidence des bactéries périphytiques aérobies, capables de dégrader les BPC, nos résultats suggèrent que la microflore anaérobie des sédiments contribue substantiellement à transformer les BPC qui contaminent le fleuve.

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APPLICATION OF SEDIMENT BIOASSAYS FOR THE ENVIRONMENT ASSESSMENT OF OCEAN DUMP SITES

A multitrophic level evaluation of sediment toxicity was conducted on sediment samples collected from the dredge and dump sites in Saint John Harbour, New Brunswick. The assays employed in this evaluation consisted of the following : Pore water and solid phase Microtox® tests (*Photobacterium phosphoreum*), Amphipod (*Amphiporeia virginiana; Rhepoxynius abronius*) survival test, Sea Urchin (*Lytechnus pictus*) fertilization test and the bacterial exoenzyme test. The results of this study were used to evaluate the application of sediment bioassays for the assessment of the environmental impact of dredged spoil disposal at ocean dump sites.

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TESSIER[1] L., VAILLANCOURT[1] G., PAZDERNIK[1] L.

ÉVALUATION IN VITRO DE LA CINÉTIQUE DU CADMIUM ET DU MERCURE CHEZ DES MOLLUSQUES D'EAU DOUCE

Nous avons comparé, en laboratoire, la cinétique d'accumulation et d'élimination du cadmium et du mercure entre le gastéropode de faible longévité *Viviparus georgianus* (Lea) et le pélécypode *Elliptio complanata* (Lightfoot) en fonction de différentes classes d'âges. Les observations préliminaires des données (concentration en métaux vs le temps d'exposition) ont démontré que l'absorption du Cd et du Hg semble suivre un patron biphasique, où un état d'équilibre est atteint après les 16 premiers jours d'exposition puis l'accumulation augmente encore jusqu'à la fin de la période d'exposition. L'élimination des métaux est aussi caractérisée par un patron biphasique. Une excrétion rapide les 4 premiers jours et une élimination plus lente le reste de la période d'élimination. Un modèle à deux compartiments a été utilisé pour évaluer, en fonction de chaque classe d'âge, les paramètres cinétiques suivants : (1) la constante du taux d'élimination, (2) la constante du taux d'absorption, (3) le facteur de bioconcentration théorique à l'état d'équilibre et (4) la demi-vie biologique des métaux étudiés. Les résultats démontrent que les gastéropodes accumulent plus rapidement les métaux étudiés que les pélécypodes. Les juvéniles possèdent un taux d'absorption plus élevé que les individus matures, et finalement le cadmium est beaucoup plus concentré, dans les mollusques étudiés, que le mercure.

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L'ABSORPTION DES MÉTAUX LOURDS CHEZ DES MOLLUSQUES SELON L'ÉQUATION D'ARRHÉNIUS

Nous avons comparé l'effet de la température sur les constantes du taux d'accumulation du cadmium et du mercure entre le gastéropode de faible longévité *Viviparus georgianus* (Lea) et le pélécypode *Elliptio complanata* (Lightfoot), en situation contrôlée. A partir de l'équation d'Arrhénius nous avons calculé l'énergie d'activation (qui caractérise l'influence de la température) de différentes classes d'âges des mollusques étudiés. Les résultats démontrent que l'influence de la température sur le taux d'absorption du cadmium et du mercure est semblable entre les différentes classes d'âges du gastéropode *V. georgianus*, l'énergie d'activation étant la même entre ces classes. Pour le pélécypode *E. complanata*, l'influence de la température sur les constantes d'accumulation du cadmium et du mercure diffère entre les classes d'âges, conséquence de l'énergie d'activation plus élevée chez les jeunes spécimens comparativement aux individus matures. Toutefois chez les moules très âgées, exposées au cadmium et au mercure, l'énergie d'activation est absente. Cette insensibilité aux variations de la température suggère que l'accumulation des métaux étudiés est sous la gouverne de processus physiques chez les vieux pélécypodes.

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TESSIER[1] L., VAILLANCOURT[1] G., PAZDERNIK[1] L.

MODELES DE PRÉDICTION DES TENEURS EN CADMIUM ET MERCURE CHEZ DES MOLLUSQUES D'EAU DOUCE

Afin d'évaluer le potentiel de deux espèces de mollusques à titre d'indicateur biologique de la pollution par le Cd et le Hg dans les écosystèmes aquatiques, des études en laboratoire et en milieu naturel ont été réalisées sur le gastéropode de faible longévité *Viviparus georgianus* (Lea) et le pélécypode *Elliptio complanata* (Lightfoot). A partir de la régression linéaire multiple nous avons généré des modèles prédictifs de la concentration en Cd et en Hg pour chaque mollusque étudié, en fonction des données obtenues au laboratoire. La concentration métallique dans la fraction aqueuse, la longueur ou la hauteur des individus et le temps d'exposition ont été sélectionnés à titre de variables indépendantes. Les résultats démontrent que le gastéropode possède le meilleur potentiel de prédiction des niveaux de Cd et Hg dans la fraction aqueuse. Les trois variables indépendantes expliquent respectivement à elles seules 72 et 83 % de la variation des teneurs en Cd et Hg. Le pélécypode présente des coefficients de régression plus faibles pour les métaux étudiés, les trois variables indépendantes retenues expliquant seulement 50 % de la variation des teneurs en Cd et 64 % de la fluctuation des concentrations en Hg. Les postulats de chaque modèle ont été validés à partir d'une étude en milieu naturel.

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QUALITÉ DE L'EAU BRUTE POUR L'ALIMENTATION EN EAU POTABLE ET PERSPECTIVES D'AVENIR

La surveillance de la qualité de l'eau des lacs, rivières et fleuve date de 25 ans au Gouvernement du Québec. Depuis 1986, l'emphase est portée sur l'évolution spatiale et temporelle de la qualité de l'eau de 25 bassins cibles afin de mesurer l'impact du Programme d'assainissement des eaux. Les paramètres analysés, au nombre de 29, regroupent des indicateurs physiques, les nutriments, les ions majeurs, les métaux, les coliformes et la chlorophylle.

Depuis 1985, le MENVIQ a aussi entrepris la recherche de micropolluants dans l'eau brute et traitée de 24 municipalités et a élargi sa couverture par la suite selon la problématique à élucider. Ainsi plus de 80 composés organiques, 13 substances inorganiques et 9 microorganismes ont été recherchés dans l'eau de plus de 300 municipalités.

Les études réalisées par Environnement Canada dans le fleuve Saint-Laurent ou à l'embouchure de ses tributaires ont aussi été mises à profit en ce qui concerne plus particulièrement les BPC, HAP, phtalates, chlorobenzènes et pesticides.

Il ressort de ces études que, sauf exception la qualité chimique de l'eau de surface avant traitement respecte les critères et normes d'eau potable pour la protection de la santé humaine.

La propension des substances inorganiques et organiques à s'adsorber aux particules et à décanter dans le milieu naturel contribuent à ce phénomène. Les substances organiques hydrophobes qui s'accumulent dans la chaîne alimentaire peuvent atteindre des seuils élevés dans la chair de poissons mais demeurent souvent non détectables dans l'eau brute. Seule l'atrazine, à l'embouchure de la rivière Yamaska, atteint momentanément des seuils supérieurs aux normes internationales. L'ajout de charbon actif granulaire dans le système de filtration au printemps assure par ailleurs l'enlèvement des pesticides.

Cependant la présence naturelle ou anthropogénique de matière organique dans les écosystèmes aquatiques, que ce soit de la couleur, des algues ou un rejet d'égout domestique, peut, en contact avec le chlore ajouté aux fins de désinfection, produire des trihalométhanes en concentrations supérieures aux normes internationales. Les trihalométhanes sont ainsi omniprésents dans l'eau chlorée et représentent lorsque la teneur de l'eau est supérieure à 3 mg/L de carbone organique dissous, la principale menace toxicologique associée à l'ingestion d'eau potable, de pair avec les autres sous-produits de la désinfection.

Dans une moindre mesure, l'impact des eaux acides sur la dissolution du plomb contenu dans les soudures des canalisations domestiques ou des entrées de service, le relarguage de HAP parfois présent dans l'enduit bitumineux de conduites d'aqueducs, ou la nature des additifs utilisés dans le traitement de l'eau potable ont fait l'objet de préoccupations de la part des organismes gouvernementaux.

A la lumière de ces études, le Québec s'apprête à adopter un nouveau règlement sur l'eau potable pour resserrer les normes des paramètres-clefs que sont la turbidité, les trihalométhanes, le plomb et le pH. Il poursuit ses travaux avec le Conseil Canadien des Ministres de l'Environnement de même qu'avec le sous-comité fédéral-provincial sur l'eau potable pour mieux orienter l'investigation des substances susceptibles d'être présentes dans l'eau de consommation. Il poursuit enfin son vaste Programme d'assainissement des eaux enclenché en 1978 à l'échelle des municipalités, des industries et des producteurs agricoles du Québec.

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THÉRIEN[1] N., MORRISON[1] K.

FLUX TEMPORELS DE MERCURE DANS LE RÉSERVOIR LA GRANDE 2 CONSIDÉRANT L'ACCUMULATION DANS LES POISSONS AINSI QUE LES DONNÉES DE RELARGAGE PROVENANT DE LA DÉCOMPOSITION DE LA VÉGÉTATION ET DES SOLS INONDÉS

L'objectif principal de cette étude était de calculer, à partir des données disponibles sur l'accumulation du mercure dans les poissons du réservoir La Grande 2, les flux temporels de mercure entre les divers compartiments biotiques de l'écosystème aquatique et d'apprécier l'importance relative de ces flux entre eux. Les calculs ont été effectués à partir des équations permettant d'établir les bilans des biomasses et des quantités de mercure en fonction du temps pour le plancton, les poissons-proies et les poissons-prédateurs. Les résultats ont indiqué que la quantité de méthylmercure relarguée dans la colonne d'eau, à partir de la décomposition de la matière organique des sols et de la végétation inondés, est plus que suffisante pour expliquer la quantité de mercure accumulée dans les poissons. Les calculs ont également montré que le cheminement du mercure par le plancton n'est pas suffisant pour expliquer les quantités de mercure emmagasinées dans les poissons-proies ni dans les poissons-prédateurs. Les calculs mirent en évidence un flux majeur de biomasse et de mercure provenant du benthos. Ces résultats apparaissent cohérents avec les données récentes portant sur les habitudes alimentaires des poissons ainsi qu'avec les concentrations typiques de mercure trouvées dans le benthos (insectes) et les poissons de l'année du réservoir La Grande 2.

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IMPACT OF HEXAZINONE AND METSULFURON METHYL HERBICIDES ON PLANKTON COMMUNITIES OF A FOREST LAKE

The impact of two herbicides on freshwater plankton communities was investigated using *in situ* enclosures. Chronic exposure to hexazinone resulted in concentration-dependent reduction in biomass of all dominant phytoplankton taxa. As derived from non-linear regression analysis, EC_{50} values had 95 % CL < 0.007 mg L^{-1}. Over 77 days of observation, no evidence of recovery in phytoplankton biomass was observed for hexazinone concentrations above 0.01 mg L^{-1}. Hexazinone induced impacts on the phytoplankton community resulted in further secondary effects in the zooplankton with EC_{50} (zooplankton abundance) of less than 0.6 mg L^{-1}. Results suggest that chronic exposure to environmentally realistic concentrations of hexazinone (i.e. ~ 1 mg L^{-1}) may result in both primary and secondary effects in freshwater plankton with recovery times proportional to exposure level. In contrast, metsulfuron methyl at concentrations as high as 1.0 mg L^{-1} (~ 40 x EEC) induced only slight, transient effects in the plankton communities. Further, study results suggest that disproportionate replication in favour of lower test concentrations and increasing the total number of test concentrations may offset problems associated with variance heterogeneity, thereby increasing confidence in toxicological endpoint predictions.

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TRAMIER[1] B.

BIODÉGRADATION DES HYDROCARBURES : CURIOSITÉ DE LABORATOIRE OU OUTIL OPÉRATIONNEL?

Le pétrole est constitué de composés en grande partie biodégradables et assimilables par l'environnement marin. C'est probablement la raison pour laquelle les grands accidents pétroliers n'ont pas conduit aux catastrophes écologiques trop rapidement annoncées.

Le processus de biodégradation naturelle du pétrole pouvait-il être accéléré et donner naissance à un outil opérationnel de lutte contre les «marées noires?»? Ce thème de recherche a été abordé depuis 1978 par les équipes environnement d'Elf Aquitaine, sous deux aspects :

- un aspect fondamental, pour mieux comprendre les mécanismes, mené dans le cadre d'un Groupement Scientifique (GS) avec le Centre National de la Recherche Scientifique,
- un aspect opérationnel destiné à mettre au point des additifs susceptibles d'accélérer le processus de biodégradation pour faciliter le nettoyage des sites atteints par une nappe de pétrole.

Les résultats de ces études qui seront décrits dans le texte définitif, montrent clairement que la biodégradation accélérée du pétrole peut être aujourd'hui considérée comme un outil opérationnel mais que, comme tout procédé biologique, cet outil a des limites et des conditions d'utilisation qui seront passées en revue, la recommandation principale étant d'utiliser systématiquement les microorganismes déjà présents dans le milieu à nettoyer. Des résultats obtenus sur des sites réels (littoral marin, nappes phréatiques) seront également présentés.

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TRANSFERT DU MERCURE SÉDIMENTAIRE À LA CHAÎNE TROPHIQUE PAR LES INSECTES

Il a été souvent rapporté que la mise en eau des réservoirs provoque une augmentation de la concentration en mercure (Hg) dans les poissons pouvant grandement dépasser les normes de santé établis par les gouvernements (0,5-1,0 mg/kg). Un élément important de la compréhension des mécanismes de transfert et de transformation du Hg, des sédiments à la chaine alimentaire réside dans la détermination du rôle des insectes aquatiques, qui par leur mode alimentaire et le développement des larves dans les sédiments, ont un contact prolongé avec le plus grand réservoir de mercure d'un système lacustre. Nos résultats démontrent que les concentrations de Hg et de methylmercure (MeHg) dans les insectes des réservoirs sont plus élevées, de l'ordre de 2 à 12 fois plus, que dans ceux d'un lac naturel. Les hémiptères, insectes brouteurs-prédateurs, ont les concentrations les plus élevées avec 1700 ppb de Hg. Ces derniers et les chironomidés (diptères) constituent une partie importante du régime alimentaire des grands corégones et pourraient, en partie, expliquer les fortes teneurs en mercure des poissons des réservoirs. La nature du substrat et sa géochimie intrinsèque semblent jouer un rôle important dans l'incorporation du Hg par les insectes alors qu'on observe des concentrations de MeHg et de carbone dans les sols inondés qui sont supérieures à celles des sédiments lacustres. De plus, on observe une augmentation du pourcentage de MeHg avec le niveau trophique des insectes.

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VAILLANCOURT[1] G., LACHANCE[1] S.

ÉVALUATION DES PERFORMANCES DE BIOCAPTEURS AQUATIQUES DANS LA MESURE DE LA BIOACCUMULATION DE SUBSTANCES INORGANIQUES ET ORGANIQUES DU HAUT ESTUAIRE SUPÉRIEUR DU FLEUVE SAINT-LAURENT (QUÉBEC)

Dans le secteur du haut estuaire supérieur du Saint-Laurent tributaire des eaux usées domestiques et industrielles charriées par le couloir fluvial, nous avons étudié la qualité du milieu en utilisant les composantes de l'environnement eau, sédiments, plantes aquatiques, lamellibranches et poissons. Toutes les substances inorganiques et organiques (sauf l'octachlorostyrène dans le support sédimentaire) ont été répertoriées dans les biocapteurs. Les taux (%) les plus élevés de plomb (58 %), de nickel (37 %) et de cuivre (44 %) ont été mesurés chez la macrophyte *Myriophyllum exalbescens* et ceux du zinc (66 %), béryllium (59 %), cadmium (50 %) et sélénium (69 %) dans l'homogénat de chair fait à partir de deux espèces de lamellibranches : *Elliptio complanata* (Ligthfoot) et *Lampsilis radiata siliquoidea* (Barnes). Les taux (%) de concentration des quatre composés aromatiques halogénés soient l'octachlorostyrène, la pentachlorobenzène, l'hexachlorobenzène et le décachlorobiphenyl ont été retrouvés par ordre d'importance, chez les lamellibranches, les macrophytes, les poissons *Perca flavescens* (Perchaude) et *Ictalurus nebulosus* (Barbotte brune) ainsi que dans les sédiments.

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VANIER[1] C., PLANAS[1] D., SYLVESTRE[2] M.

DEVENIR DES BIPHÉNYLS POLYCHLORÉS DANS LES SÉDIMENTS

L'objectif de cette étude est d'évaluer le devenir spatial et temporel des biphényls polychlorés (BPC) dans les sédiments du lac Saint-François (fleuve Saint-Laurent). Les sédiments d'une station fortement contaminée et de deux stations en aval ont été échantillonnés à une profondeur de 27 cm et analysés par strates de 3 cm.

Dans les stations en amont, la couche 18-21 cm présente les concentrations les plus élevées en BPC (respectivement 8,2 et 2,0 $mg.kg^{-1}$ de sédiments frais); la station la plus en aval présente un léger pic de BPC entre 3 et 6 cm (1,1$mg.kg^{-1}$, poids frais). L'analyse de correspondance basée sur le % des isomères (di-, tri-, tétra-, penta-, hexa-, hepta-, et octachlorobiphényls) dans les sédiments et différents Aroclors montre une association entre la station la plus contaminée et l'Aroclor 1242, alors que les deux autres stations sont plutôt associées aux Aroclors 1248 et 1254. Dans le cas de ces stations, des sources de contamination différentes ou un appauvrissement progressif en BPC plus volatiles et biodégradables peuvent expliquer leur association aux isomères plus chlorés (plus de 3 chlores).

D'autre part, les couches profondes de la station la plus contaminée montrent un enrichissement en congénères faiblement chlorés (particulièrement # 15, IUPAC). Ce phénomène, absent dans les autres stations, suggère un processus de déshalogénation.

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ACUTE TOXICITY OF OIL SANDS WASTEWATER : A TOXIC BALANCE

The goals of this study were to 1) to identify and determine the relative importance of the acutely toxic fractions of the wastewater from oil sands extraction using a bioassay directed Toxicity Identification Evaluation (TIE) and to use these data to construct a toxic balance, and 2) to determine whether the same fractions were acutely toxic to Microtox®, *Daphnia* and rainbow trout. Samples of extraction wastewater were obtained from the tailings pond at Syncrude Canada Ltd. during the summers of 1991 and 1992. The samples were centrifuged to remove suspended solids and the toxicity of the supernatant was evaluated. Seven manipulations, each of which was designed to remove a different class of compounds, was performed on the wastewater samples. A complete TIE was performed with Microtox®. All toxicity of the surface tailings water to Microtox® was removed by precipitation of organic acids or by removal of nonpolar organics. These results suggest that the toxic fraction was a surfactant. Volatiles accounted for 15 % of the toxicity of interstitial pore water. However all toxicity was removed by precipitation of organic acids or by the removal of nonpolar organics. Organic acids accounted for all the toxicity of tailings pond water in *Daphnia* and rainbow trout. Acute toxicity of tailings pond water to *Daphnia* was 0.4 times less sensitive and rainbow trout were 3 times more sensitive than Microtox®. These differences in relative sensitivity of test organisms to the toxic fraction indicate the importance of using more than one test organism to evaluate acute toxicity.

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BASELINE HUMAN HEALTH RISK ASSESSMENT FOR THE CONSUMPTION OF LOBSTER FROM HALIFAX HARBOUR

A baseline human health risk assessment on the consumption of lobster from Halifax Harbour was completed as part of the evaluation of a proposed primary sewage treatment plant (STP) for the Halifax-Dartmouth metropolitan area. The risk assessment was based on a thorough review of available scientific literature, the available data on the quality of lobster tissue (meat and hepatopancreas) from Halifax harbour, and the data and conclusions of other researchers undertaking complementary studies on the STP in Halifax Harbour. According to Health and Welfare Canada (HWC) «...no restriction in the consumption of lobsters from the Halifax harbour is considered warranted at this time» (Kirkpatrick 1990). The analysis in this assessment did not contradict HWC's conclusion. However, it showed that exposure data, i.e. who consumes the lobsters, is an important data gap. It also revealed that risk managers must rely heavily on their judgement of the strength of experimental animal evidence to predict adverse human health effects from polychlorinated biphenyls and polycyclic aromatic hydrocarbons. While polychlorinated biphenyls are highly regulated and should decline as a risk factor in the Canadian Environment, polycyclic aromatic hydrocarbons are unlikely to decrease in the near future. From the standpoint of the protection of human health, the depth of relevant toxicological data on this group of compounds does not appear to reflect the apparent concern for their toxicity, as expressed by current suggested marine environmental quality guidelines.

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A NEW SAMPLER FOR COLLECTING DRY ATMOSPHERIC DEPOSITIONS OF ORGANIC CHEMICALS

Studies conducted in Saskatchewan and elsewhere have demonstrated the atmospheric transport of agricultural pesticides and other organic contaminants and their deposition into aquatic ecosystems. To date these studies have focused on the ambient concentrations in the atmosphere and on quantities in wet precipitation. To measure the dry deposition of organic chemicals, we designed a new sampler, which uses a moving sheet of water to passively trap dry particles. Chemicals collected can be related to the materials entering aquatic systems. The new sampler, which is currently under the patenting process, will be described. Preliminary testing data reported and implications for aquatic ecosystems will be discussed.

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GEOCHEMICAL PARTITIONING OF COPPER IN AN ARTIFICIAL OXIDE-ORGANIC SEDIMENT

Aquatic systems constitute the largest sink for heavy metals mobilized by anthropogenic activities. Currently, a prevalent paradigm for metal toxicology and biogeochemistry is that geochemical partitioning between solid and solution phases controls bioavailability of metals. We have investigated the effects of competition between discrete geochemical fractions (organic matter and Fe oxides), and between metals (Cd and Cu), on the partitioning of sedimentary copper and its subsequent bioavailability to an aquatic plant. Organic matter and a synthesized Fe oxide, ferrihydrite, were added singly and in combination to a series of sand sediments, which were then dosed with environmentally relevant concentrations of Cd and Cu and planted with *Oryza sativa*. Results presented here will focus on the geochemical partitioning of Cu. The oxide bound negligible amounts of either metal, even in the absence of organic matter. Organic matter controlled copper partitioning. As organic matter concentrations increased, operationally defined, leachable copper decreased and organic associated copper increased. There were no significant differences in partitioning observed between the Cu and Cu + Cd treatments. The absence of a competitive effect of Cd on Cu partitioning suggests surface site specificity in cation complexation. The lack of any significant oxide sorption of metal during the course of the experiment was an unexpected result. We will discuss a possible mechanism explaining this result.

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THE STATUS AND FUTURE OF MARINE MICROSCALE TOXICITY TESTS

Microscale approaches for assessing marine ecotoxicity have rapidly developed over the past 10-15 years. They currently are contributing to problem identification, research, assessment, control and monitoring of marine pollutants in many countries. This paper describes marine microscale testing, emphasizing innovative exposure and effects techniques, recent progress with species from bacteria (e.g. Microtox®) to teleosts (embryo assays), especially with mero- and holo-zooplankton, and applications in sediment toxicology, QSAR evaluations, TIE's of effluents and sediments, and effects monitoring. Novel techniques, such as microinjection of contaminants, the measurement of limb regeneration, and cryogenic preservation, are described. Limitations of approaches to date exist; they include seasonal gamete availability, uncertain taxonomy, and approximate estimation of contact exposures and chemical partitioning rates. Exceptional studies across the taxa are summarized, with special emphasis on techniques with species of high commercial and/or ecological importance. Many research challenges remain with marine microscale assays, from understanding the basis for comparative sensitivities of different species and life stages, to developing better techniques of culture and handling. Such assays offer many advantages and opportunities for research and pollutant regulation; as part of hazard assessment schemes, they promise to be a focus of much effort throughout the 1990's in the continued fight to protect and conserve marine ecosystem health.

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CHRONIC TOXICITY OF COPPER TO LARVAL FATHEAD MINNOWS IN SOFT WATER

We examined the chronic toxicity of copper to fathead minnows in Dickie Lake, a high DOC, soft water lake (DOC, 5.8 mg/L) located on the Precambrian Shield. Other important modifiers of copper toxicity were held constant at ambient lake levels (pH, 6.0; calcium, 2.5 mg/L). Toxicity tests were carried out in a class 1000 clean lab located at the Dorset Research Centre, MOEE, Dorset, Ontario. Ten larval fish were continuously exposed to each of 6 Cu concentrations (1.8, 3.2, 5.6, 10, 18, 32 µg Cu/L) plus a control for 21 d. Mortality was recorded twice a day and total length and dry weight were measured at the end of the test. Survivorship was the most sensitive parameter measured; the lowest observed effect concentration (LOEC) was 3.2 µg Cu/L. Both total length and dry weight were less sensitive than survivorship (LOEC was 10 µg Cu/L for each). We anticipate that the chronic toxicity of copper to the larval fathead minnow in a low DOC lake (Margaret Lake; DOC, 2.0) will be lower than the effects observed in the high DOC lake. These results indicate that copper concentrations at or below current water quality objectives may have deleterious effects on fathead minnows.

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BEHAVIOURAL RESPONSES OF RAINBOW TROUT TO INDUSTRIAL EFFLUENTS

Locomotor responses of individual juvenile rainbow trout, *Oncorhynchus mykiss*, to industrial effluent samples and to relevant toxicants were monitored by two automated laboratory methods. In one method, the avoidance/preference responses to toxicant, were monitored in a steep gradient tank while the fish were exposed to sequentially increasing concentrations of each of copper and sodium lignosulphonate. The avoidance threshold, estimated by change point analysis, for copper was 23.57 μg/L total copper. No significant response to sodium lignosulphonate was found. In the other method, rainbow trout were exposed to temporal gradients of several toxicants (the fish were deprived of spatial clues by introduction of the toxicant to the tank as a whole). Guaiacol, p-chlorophenol, sodium lignosulphonate and two pulp mill effluent samples caused significant changes in activity levels of the fish while abietic acid, copper, and potassium chromate did not. The difference in responses between the two methods of exposure may help explain mechanisms and the differences between laboratory and field avoidance/preference observations.

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PREDICTIVE QUANTIFICATION OF TRACE METAL DYNAMICS AND PARTITIONING IN PARTICULATE MATTER

A quantitative method of assessing trace metal concentrations in environmental particles has been developed. A simplified system consisting of relatively homogeneous kaolinite particles was implemented and a mass balance approach was applied to assess trace metal adsorption/desorption kinetics under a range of conditions. Various pHs, particle densities, and trace metal concentrations were studied to test the proposed method. The results suggest that it is possible to determine trace metal levels associated with the particulate phase in aquatic systems and to predict partitioning between water and suspended particulate matter. The relevance of this approach to trace metal bioavailability in aquatic organisms will be discussed.

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^{32}P-POSTLABELLING ANALYSIS OF AROMATIC DNA ADDUCTS IN LIVER OF WHITE SUCKER (CATOSTOMUS COMMERSONI) AND CARP (CYPRINUS CARPIO)

Using the highly sensitive ^{32}P-postlabelling technique, we have determined the DNA adduct levels in liver tissues of white sucker and carp from PAH-contaminated and uncontaminated St. Lawrence and St. François rivers, respectively. The behavior of the adducts in the diagonal radioactive zone is consistent with their identification as nucleotide adducts of a variety of bulky hydrophobic aromatic environmental compound. Total adduct levels found in all fish species ranged from 63 to 142 adducts per 10^9 nucleotides. Detectable levels of exogenous aromatic DNA adducts due to PAH-contamination from St. Lawrence river found in white sucker, varied from 55 to 85 adducts per 10^9 nucleotides. Dominant features of the fish DNA adducts were the high variation in total adducts level among individuals in the same population and a chromatographic pattern species specificity.

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PROCEEDINGS OF THE ROUND TABLE ON BIOMARKERS IN ECOSYSTEM AND HUMAN HEALTH ASSESSMENT

P.V. Hodson and J-G. Bontoux, Co-chairs

The Round Table on Biomarkers in Ecosystem and Human Health Assessment was organized to review and discuss the use of biomarkers of toxicity and chemical exposure in the assessment of human and ecosystem health. The purpose was to exchange ideas, to identify areas common to human and ecosystem health assessment, to raise critical scientific issues, and to develop research needs to resolve these issues. The Round Table was held as part of the *20th Aquatic Toxicity Workshop* in Quebec City, October 18-20, 1993. Recent advances in the development and use of 'biomarkers' in health assessments were presented by two keynote speakers, followed by responses from Round-Table participants and discussions with the general audience.

Ecosystem Panel

Chris D. Metcalfe **(keynote speaker)**
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Paule Vasseur
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Phil Spear,
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Pierre Deschaux,
U.E.R. des Sciences,
LIMOGES

Kelly R. Munkittrick,
GLLFAS, Fisheries and Oceans
Burlington

Dr. Peter Hodson
National Water Research Institute
Environment Canada, Burlington

Human Health Panel

Jean-Marie Haguenoer **(keynote speaker)**
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France, LILLE

Jean-Géraud Bontoux,
Dépt. Sciences de l'Environnement et Santé
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Dr. Sarah England
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Dr. A. Gilman
Environmental Health Directorate
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Mr. Mark Feeley,
Foods Directorate,
Health and Welfare Canada, Ottawa

The Round Table was divided into two sessions, a morning session on biomarkers in ecosystem studies, and an afternoon session on biomarkers in human health studies.

Biomarkers in studies of ecosystem health

The session keynote speaker was Dr. Chris Metcalfe, Trent University, who defined biomarkers as indicators of exposure or effects of chemicals at the sub-organismal or organismal level. He illustrated this definition by describing the use of biomarkers for the exposure and effects of polynuclear aromatic hydrocarbons (PAH) on both freshwater and marine fish species. These biomarkers included measurements of increased activity of mixed function oxygenase (MFO or P450 enzyme system), bile concentrations of PAH metabolites, frequency of micronuclei, concentrations of DNA adducts and tumour frequency. Effectively, for PAH, all the classical stages in carcinogenesis have been demonstrated.

These indicators have been applied to studies of species that are commercially important and have been used to demonstrate the success of remediation. However, regulatory activity arising from studies using biomarkers respond more to perceived relevance to human health and to economic importance, than to ecosystem health.

Research needs that are most important are the development of 'norms' for measured responses and the range of responses that represent acclimation to chemical exposure rather than toxicity.

The responses of members of the Round Table were both supportive and critical of the use of biomarkers. Marc Lafaurie reported similar success in the application of biomarkers to studies of fish in areas impacted by urban development and industrial pollution in the Mediterranean However, he emphasized that at the cellular and organismal level, the molecular basis for a biomarker response must be known to understand the relationship between cause and effect. A wider definition of biomarkers to encompass 'changes to biological systems' was proposed (Phil Spear) to encompass responses at the population and ecosystems level, as well as responses of behavior, morphology and molecular biology. Since responses at the sub-organismal level precede those at the population or ecosystem level, biomarkers should not only indicate the status of a system, but also act as a sensitive alarm of possible future harm.

A more fundamental criticism from Kelly Munkittrick was that indicators of exposure were often misinterpreted as indicators of effects, when the link between the exposure indicator and important effects (e.g. reproduction) were not at all understood. Furthermore, 'ecosystem health' is a term without a clear definition. Hence the use of organismal level biomarker to indicate 'ecosystem health' is not valid - studies should proceed from observations at the ecosystem level, the 'top-down' approach. There is the additional problem that methods for many biomarkers are not well worked out and may contribute to considerable variability among and within studies.

Paule Vasseur responded that indicators of exposure and toxicity are not necessarily separate; the dividing line is the threshold of response when adaptation to exposure becomes pathological. The question of whether toxicity follows a biomarker response becomes then a question of probabilities. To adequately describe and predict effects, a multiplicity of biomarkers is required, applied to studies of ecosystems by multidisciplinary teams.

The importance of an 'epidemiological approach' to studies of chemical effects on wild fish was emphasized by Peter Hodson. Physiological and biochemical responses of fish, often cited as biomarkers of the effects of pulp mill effluents, may also be sensitive to natural gradients in the absence of effluents. While the effluents may cause some of these responses, it is clear that cause-effect relationships are not unequivocal, which reduces the value of these responses as biomarkers without solid epidemiological sampling designs.

Pierre Deschaux pointed out that the immune system was an excellent source of biomarkers, one that was often overlooked. He identified both specific and non-specific, cellular and non-cellular immune responses that are activated by exposure to pathogens, and that can be stimulated or repressed by exposure to chemical agents.

The remarks of the Round Table participants were followed by some interesting comments and questions from the audience. The suggestion was made that policy makers be included in planning effects studies, to ensure that relevant questions were asked and that appropriate biomarkers were included. The idea of an early warning role for biomarkers was also re-stated, with the view that the possible consequences of biomarker responses should be interpreted in terms of probability functions for specific toxic effects. Risk estimates would generate a more suitable response from management and policy makers, analogous to the reactions of smokers to information on cancer risks. The philosophy of applying biomarkers was also questioned - What do biomarkers show that is not demonstrated by chemical analyses? Should ecosystem 'health' be expressed in terms of specific chemical criteria? Round Table members responded that biomarkers offered significant cost advantages, and that they represented the integrated response of organisms to all similar chemical stressors, and not simply the consequences of one specific analyte. However Harrish Sikka (SUNY) emphasized the specificity of certain biomarkers, using the example that DNA adducts are not universally generated by all contaminants; rather, they are restricted to specific PAH compounds. This, in fact, is one of their advantages.

Kats Haya (St. Andrews) questioned whether the purpose of measuring biomarkers was clear, and the panel responded that biomarkers could give clear information on exposure and toxicity, specifically sublethal and chronic effects. Paule Vasseur presented some results demonstrating that a strong relationship could be found between risk of hepatocarcinoma and liver DNA adduct levels in some experimental species exposed to a carcinogen; hence in these cases, the adducts can be used to predict risk, i.e. the likelihood of a given frequency of cancer. Though these results are promising, the prediction is not so simple for the following reasons: Firstly, adducts-risk correlations were shown to differ among species, the organ and the carcinogen being considered; secondly, the estimation of cancer risk will be more complicated under environmental conditions than in experiments because populations are exposed to mixtures of potentially carcinogenic pollutants leading to the formation of a myriad of various adducts whose combined effects are unknown.

While there appeared to be frequent and strong criticisms of the application of biomarkers to detecting problems of chemical contamination in wild fish populations, there was at the same time a strong interest in the concept due to the perceived potential benefits. The criticisms effectively highlighted some very important research needs, among them:

1. Definition of 'normal' for responses of fish populations adapted to a wide range of natural environments, and undergoing dramatic shifts in physiology, habitat selection and chemical exposure during their lifetime.

2. Development of an understanding of the mechanisms linking chemical exposure to biomarker responses, and biomarker responses to effects on rates of reproduction, growth, disease and mortality of fish populations.

3. Development of 'standard methods' to allow comparison of results among studies and to avoid biassed or confounded conclusions.

4. Distinguish between responses that demonstrate chemical exposure and adaptation to low level stress, and responses that represent toxicity. What are the thresholds of response where the transition occurs?

5. The expression of results in terms of risk statements, i.e. the probability of a specific toxic effect predicted by a given response level of a biomarker. This will require quantitative experiments in which the link between biomarker response and toxicity is well understood.

6. The identification and development of biomarkers through studies of impacted populations (the 'top-down' approach). By studying the mechanisms by which 'important' (relevant) effects such as reproductive impairment are induced by chemical toxicity, suitable biomarkers can be chosen from the chain of events between chemical exposure and population level impacts.

7. The development of sampling designs for valid epidemiological studies to relate cause and effect, taking into account bias from natural stressors.

8. The application of immunology to studies of chemical effects on fish.

Biomarkers in studies of human health

Dr. Jean-Marie Haguenoer was the keynote speaker for the afternoon session on biomarkers in monitoring human heath. The use of biomarkers is particularly well developed for the control of human health in the work-place. This strategy has lead to an increasing surveillance of populations exposed to environmental risks associated with industrial, food, microbial or viral exposures. Indicators include measures of external dose or environmental exposure, internal dose (e.g. tissue concentrations), and early markers of toxicity, including measurements ranging from psychomotor testing to physiological responses. With humans, there is considerably more known about 'normal' responses and factors that contribute to sensitivity to chemical exposure. Hence, application of 'biomarkers' can be enhanced through measures of these related life-style and environmental factors. Much of the concern expressed about the adequacy of epidemiological designs and the specificity of biomarkers in the assessment of aquatic ecosystem health was also expressed about human health assessment. However, the science of human epidemiology seems much more advanced than eco-epidemiology, particularly in industrial settings.

In response to the keynote speech, Jean-Geraud Bontoux pointed out that epidemiology dealt more with populations than with individuals, an emphasis which is even stronger in eco-epidemiology where the lives of individual organisms are not the focus of studies. Hence the nature of measurements and study designs should reflect the population emphasis.

The importance of developing biomarkers from known mechanisms of toxicity was emphasized by Pierre Ayotte, who used the caffeine breath test as an example of a validated biomarker of chemical exposure and metabolism (via the MFO system). The question of experimental proof must be applied to all proposed biomarkers. Andrew Gilman pointed out the difficulty of detecting the effect of multiple chemicals at low level exposures. He felt that current biomarkers were not well-enough developed to distinguish disease states from individual variability. Too little was known about dose-response and the relationship of biomarker responses to disease. Research is needed to specifically address these issues, and to develop biomarkers within well-defined populations. Budgets should be put into a few intensive research studies rather than into widespread monitoring.

Mark Feeley supported some of the main points made by Jean-Marie Haguenoer, emphasizing the importance of exposure-response relationships, the definition of 'normal', and the relationship of biomarkers to 'health'.

At the end of the day, Sarah England returned the discussion to one of the points raised by Chris Metcalf. i.e. the relevance of much of the regulatory program to ecosystem health. Since regulatory actions are often concerned with human health, employment, and the standard of living, it is difficult to sell regulators on the importance of indicators of ecosystem health. Ecosystem health programs must start with a clear agenda and, as Jean-Geraud Bontoux concluded, the relationships between ecosystem and human health monitoring were not at all clear and would require considerably more interactions among scientists in both disciplines. Overall, the major points emerging from the afternoon round table were:

1. The use of biomarkers is well developed for the control of human health in occupational settings and for detecting and preventing atmospheric transport of contaminants; use in surveillance of specific industrial populations is increasing.

2. There appears to be a parallel development of biomarkers in the assessment of human and ecosystem health, with identical debates on validity and utility as indicators of exposure and/or effect;

3. Methods exchanges between specialists in human and animal health is in the formative stage. Experience in human epidemiology appears better developed than in ecosystem epidemiology. However, even if sampling cannot be carried out in the same way by the two disciplines, the interpretation of the statistical significance of results in ecotoxicology would be enriched in exchanges with experts in human epidemiology.

4. Species-specific responses can greatly limit the extrapolations of data on responses of aquatic biota to man. However, research on species that are particularly sensitive can lead to the development of very sensitive early warning indicators

5. Research on biochemical biomarkers in ecotoxicology can generate new knowledge on the mechanism of detoxification and ultimately have applications in human health protection

6. The development and extent of ecosystem studies may be limited by public opinion that is more sensitive to questions of sanitation and human health than to protection of natural environments.

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PRIX DES MEILLEURES PRÉSENTATIONS D'ÉTUDIANTS/ BEST STUDENT PRESENTATIONS AWARDS

1) Meilleure affiche/ best poster:

C. Flessas

"Analyse des facteurs biotiques et abiotiques influensant la bioaccumulation des métaux lourds chez *Bithynia tentaculata* (*mollusca: gasteropoda*) du lac Saint-Louis"

Université de Montréal, Département des Sciences biologiques, Montréal.

2) Meilleur exposé/ Best briefing

M.E. McMaster

"Evaluation of the steroid biosynthetic capacity of ovarian follicules from white sucker exposed to bleached Kraft mill effluent (BKME)"

University of Guelph, Department of zoology, Guelph.

JURY D'ÉVALUATION/ EVALUATORS

B. Pinel Alloul (présidente/chair)	Université de Montréal, Département des sciences biologiques, Montréal
C. Blaise	Environnement Canada, Centre Saint-Laurent, Longueuil
R. Chassé	Ministère de l'Environnement et de la Faune du Québec Sainte-Foy
F. Fick	Université d'Ottawa, Département de biologie, Ottawa.
P.V. Hodson	Environnement Canada, Burlington
L. Martel	Ministère de l'Environnement et de la Faune du Québec Sainte-Foy
H. McCormick	United States Environmental Protection Agency, Duluth
J. Pelerin	Université du Québec à Rimouski, Département d'océanographie, Rimouski
D. Planas	Université du Québec à Montréal, Département des sciences biologiques, Montréal
K. Solomon	Guelph University, Guelph

ACTIVITÉS PARALLÈLES/ PARALLEL ACTIVITIES

Lundi/ Monday	**18 octobre/ October 18th**
19:00 - 24:00	Rencontre du Comité permanent international des colloques de toxicologie aquatique/Meeting of the international Aquatic Toxicity Workshops. Responsable/Convenor: A. Niimi*
Mercredi/Wednesday	**20 octobre/ October 20th**
08:30 - 12:00	Rencontre des représentants de l'AQ-CQ pour les laboratoires de toxicologie de l'Association canadienne des laboratoires d'analyse en environnement/Meeting of the Canadian Association of Environmental Aquatic laboratories: toxicological laboratory QA/QC representatives. Responsable/Convenor: R. Scroggins*
13:30 - 17:00	Détermination des mesures terminales de toxicité: Statisques et programmes informatisés/ Toxicity endpoint determinations: Statistics and computer programs. Responsable/Convenor: J. Miller*
14:00 - 17:00	Réunion du comité du manuel d'interprétation de la toxicité/Toxicity Interpretation Manual Steering Commitee Meeting. Responsable/Convenor: B. Taylor*
Jeudi/Thursday	**21 octobre/ October 21st**
09:00 - 17:00	Rencontre du groupe intergouvernemental de toxicologie aquatique/ Meeting of the Intergovernmental Aquatic Toxicity Group. Responsable/Convenor: R. Scroggins*
08:30 - 16:30	Symposium sur le suivi aquatique des effets environnementaux/ Symposium on Aquatic Environmental Effects Monitoring. Responsable/Convenor: S. Forsyth*
07:30 - 21:00	Excursion post-colloque aux baleines/Post-Workshop Whales excursions Responsable/Convenor: R. van Coillie*

* Pour les comptes rendus de ces activités parallèles, veuillez contacter le responsable dont l'adresse figure dans la liste des participants./ For the proceedings and/or informations on the parallel activities, please contact the convenor at his address mentionned in the participants list.

LIST OF PREVIOUS WORKSHOP PROCEEDINGS

The Proceedings of the Annual Aquatic Toxicity Workshops have been published as a serie of technical reports listed below. Copies of recent proceedings may be available from A. Niimi, Continuity Chairman, Aquatic Toxicity Workshop, Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6. Copies of most proceedings are available for a charge from Micromedia Limited, 165 Hotel de Ville, Place du Portage, Hull, Quebec J8X 3X2, (819) 880-9928. Their catalog numbers (MLCN) are listed below where applicable.

Proceedings of Twentieth Annual Aquatic Toxicity Workshop: October 17-21, 1993, Quebec City, Québec. Edited by R. van Coillie, Y. Roy and collaborators Can. Techn. Rep. Fish. Aquat. Sci.: 331 p.

Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore Can. Tech. Rep. Fish. Aquatic. Sci. 1942: 489 p.

Proceedings of the Eighteenth Annual Aquatic toxicity Workshop: September 30-October 3, 1991, Ottawa, Ontario. Edited by A.J. Niimi and M.C. Taylor. Can. Tech. Rep. Fish. Aquat. Sci. 1863: 381 p. (MLCN: 97-6/1863).

Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, BC. Edited by P. Chapman, F. Fishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuk and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p (MLCN: 91-06176).

Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. van Coillie, A. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p. (MLCN: 90-0185).

Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A. J. Niimni and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p. (MLCN: 88-04587).

Proceedings of the Thirteenth Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p. (MLCN: 88-01709).

Proceedings of the Twelfth Annual Aquatic Toxicity Workshop: November 5-8, 1985, Thunder Bay, Ontario. Edited by G.W. Ozburn. Can. Tech. Rep. Fish. Aquat. Sci. 1462: 229 p. (MLCN: 86-5828).

Procedings of the Eleventh Annual Aquatic Toxicity Workshop: November 13-15, 1984, Vancouver, BC. Edited by G.H. Green and K.L. Woodward. Can. Tech. Rep. Fish. Aquat. Sci. 1480: 330 p. (MLCN: 86-1103).

Proceedings of the Tenth Annual Aquatic Toxicity Workshop: November 7-10,1983, Halifax. Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 475 p (MLCN: 86-1103).

Proceedings of the Ninth Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mackay. Can. TEch. Rep. Fish. Aquat. Sci. 1163: 243 p. (MLCN: 84-3262).

Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish Aquat. Sci. 1151: 255 p. (MLCN: 83-2515).

Proceedings of the Seventh Annual Aquatic toxicity Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert, M. Speyer and R. van Coillie. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p. (MLCN: 82-0070).

Proceedings of the Sixth Annual Aquatic Toxicity Workshop: November 6-7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp., S.L. Leonard and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p. (MLCN: 81-1492).

Proceedings of the Fifth Annual Aquatic Toxicity Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann. Fish Mar. Ser. Tech. Rep. 862: 342 p. (MLCN: 80:4061).

Proceedings of the Fourth Annual Aquatic Toxicity Workshop: November 8-10, 1977, Vancouver, BC. Edited by J.C. Davis, G.L. Greer and I.K. Birtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p. (MLCN: 80: 4022).

Procedings of the Third Annual Aquatic Toxicity Workshop: November 2-3, 1976, Halifax, Nova Scotia. Edited by W.R. Parker, E. Pessah, P.G. Wells and G.F. Westlake. Environ. Prot. Ser. Tech. Rep. EPS-5-AR-77-1.

Proceedings of the Second Annual Aquatic Toxicity Workshop: November 4-5, 1975, Rexdale, Ontario. Edited by G.R. Craig, Ontario Ministry of the Environment.

Compendium of aquatic toxicity studies in Canada, 1974. Unpublished Report, Freswater Institute, Winnipeg, Manitoba.

LISTE DES PRÉSIDENTS ANTÉRIEURS/LIST OF PREVIOUS CHAIRS

Le 20e anniversaire des colloques de toxicologie aquatique a été célébré à Québec au banquet du 19 octobre 1993. A cette occasion, on fit une rétrospective et on donna des surnoms aux présidents antérieurs./ The Aquatic Toxicity Workshop's 20th anniversary was celebrated on October 19th, 1993 at the banquet; an historical review was done and a nick name was given to each previous workshop's chairs.

Numéro du colloque/ Workshop number	Lieu/Site Année/Year	Président antérieur/ Previous chair	Surnom/Nick name
1	Winnipeg (1974)	J. Klaverkamp	Buffalo Monk
2	Toronto (1975)	G.R. Craig	Tower Monk
3	Halifax (1976)	P. Wells	Bluenose Monk
4	Vancouver (1977)	J.C. Davis	Salmon Monk
5	Hamilton (1978)	P.V. Hodson	Pollution Monk
6	Winnipeg (1979)	J. Klaverkamp	Prairie Double Monk
7	Montréal (1979)	N. Bermingham	Energetic Monk
8	Guelph (1981)	K. Solomon	Academic Monk
9	Edmonton (1982)	W.C. Mackay	Oil Monk
10	Halifax (1983)	P. Wells	Lobster Double Monk

Numéro du colloque/ Workshop number	Lieu/Site Année/Year	Président antérieur/ Previous chair	Surnom/Nick name
11	Vancouver (1984)	G.H. Green	Remember
12	Tunder Bay (1985)	G.W. Osburn H. McCormick	Thunder Monk Uncle Sam Monk
13	Moncton (1986)	J.S.S. Lakshminarayana	Shrimp Monk
14	Toronto (1987)	G. Westlake	Daphnid Monk
15	Montréal (1988)	R. van Coillie	Good living Monk
16	Winnipeg (1989)	S. Leonard	Freshwater Nun
17	Vancouver (1990)	P. Chapman	Globetrotter Monk
18	Ottawa (1991)	M.C. Taylor	Flying Nun
19	Edmonton (1992)	E.G. Baddaloo	Cow Boy Monk
20	Quebec city (1993)	R. van Coillie	Tutti Frutti Double Monk

and...

continuous chair, A. Niimi, Burlington, Pope Monk